EFFECT OF SILVER AND ZINC OXIDE NANOPARTICLES ON THE PHYSIOLOGY AND GERMINATION OF WHEAT SEED

By

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FINAL APPROVAL

It is certified that the contents and form of the thesis entitled **"Effect Of Silver And Zinc Oxide Nanoparticles on the Physiology and Germination Of Wheat Seed"** submitted by Mr. Rafiq Muhammad have been found satisfactory for the requirement of the degree of MS/M.Phil in Bio Science.

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DEDICATED

To

My Mother

A strong and gentle soul who thought me to trust in Allah, believe in hard work and that so much could be done with little.

My Father and Brothers

For earning an honest living for us and for supporting and encouraging me to believe in myself.

My Sister

Respected, loveable and humble sister (Kamia Saida) who stood beside me at every moment in my life

My Son

Muhammad Hasnain for bringing so much joy to my life

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, Except where otherwise acknowledged and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.

Date 29/09/2017

RAFIQ MUHAMMAD

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growth (Prasad *et al.*, 2012, Sedghi *et al.*, 2013, Ramesh *et al.*, 2014). Until now most observation have pointed the relation of ZnO NPs with phyto toxicity as observed germinating seed (López-Moreno *et al.*, 2010). The main role of soil properties in affecting Zn bio availability from ZnO nanoparticles is little known. Plants toxicity was not studied in soybean grown in an organic farm soil where the plants absorbed Zn from ZnO NPs (Priester *et al.*, 2012). In onion (*Allium cepa*) show that lower concentration of ZnO NPs exhibited positive effect on seed germination. But higher concentration is can negatively affect seed germination. The impact of zinc oxide nanoparticles on seed sprouting depends on the concentration of nanoparticles and different from plants to plants. (Laware & Raskar, 2014). Plants that growing with ZnO NPs would be responding, to both the NPs and Zn ions released from the NPs. There are discussions on relative effectiveness of the nano sized particles versus the solubilized ions in eliciting cellular responses. (Fernández *et al.*, 2013, Ma *et al.*, 2013).

1.5. Why Ag and ZnO

For the present study, Ag and ZnO nanoparticles were selected for the application on common wheat *(Triticum aestivum)* on the basis information available in literature and work performed at SA-SIRBS, IIUI Islamabad. We revealed that among the nanoparticles, Ag and ZnO nanoparticles are widely used in numbers of applications in plants. Ag and ZnO are more compatible for application of plants growth and physiology. Hence in current research project, we have studied the impact of Ag NPs and ZnO NPs on seed germination, root/shoot length along with some physiological parameters of *Triticum aestivum* (wheat plant).

1.6. Significance of study

Scientists have worked on different nanoparticles, and studied their effect on plants development and growth and also observed the effect of NPs on physiology and seed germination of plants. To studies these parameters, scientist applied different concentration of nanoparticles. (López-Moreno *et al.*, 2010, Krishnaraj *et al.*, 2012, Pandey *et al.*, 2014)

There is little information available related to the influence of nanoparticles on crop plants. Therefore this study was designed to identify the positive and negative impact of nanoparticles on plants development and growth.

1.7. Objectives

Keeping in view all the knowledge gained from literature related to nanoparticles and plants, it was hypothesized that the application of nanoparticles with different concentration may promote or inhibit plant growth and development, depending on their concentration. In this context, the objectives of the present study were:

- Impact of Silver (AgNPs) and Zinc nanoparticles (ZnNPs) on Wheat *(Triticum aestivum)* seed germination.
- Effect on root-shoot elongation of wheat plants.
- Promontory or inhibitory effects of various concentrations of Zn nanoparticles and AgNPs on growth and development.
- Effect on chlorophyll contents by using various concentrations of Silver (Ag NPs) and Zinc Nanoparticles (Zn NPs).
- Effects on primary metabolite contents.

1.8. Scope of the study

The scope of the study is providing an evaluation of Ag and ZnO nanoparticles uptake and growth performance of *Triticum aestivum* plants. This study will help to give new information about wheat plants responses to different concentration of Ag and ZnO nanoparticles. Particular nanotechnology approaches may be applied to improve the nutrient availability to food crops especially soluble zinc oxide and silver

Chapter. 02

LITERATURE REVIEW

Main focus of this chapter to provide the detailed information related to applications of nanoparticles on plants. Their possible mechanisms and their beneficial as well as harmful effect on plants development and growth.

2.1. Plants and nanoparticles Interaction

The advancement of nanotechnology and its linkage with biology improve its application in different fields. Application of nanomaterials in different area of research concentrated to improve effectiveness and productivity. The nanoparticles used in crops production and agricultures production (Nair et al., 2010, Sharon et al., 2010).

Recently nanoparticles are mostly used in advance technology in agriculture and others field of biology which increase the chance of exposure and uptake of these materials in plants. Plants are most imported components of ecosystems and provide possible pathways for nanoparticles transport into foods chain and ecosystems. Therefore plants provide a route to come into the food web through bioaccumulation. It was studied that silver nanoparticles were transformed into hornworm from tobacco (Judy *et al.*, 2010).

Nanoparticles present in environment may be accumulated in different parts of the plants and possible way through which nanoparticles enter to plants is roots and stomata of leaves (Eichert *et al.*, 2008). The common way of plants vulnerability to nanoparticles is wastewater sludge used in agriculture field (Lee *et al.*, 2010). The direct application of nanoparticles is a new concept and need further explanation regarding their safe used and their outcome production. The huge used of nanoparticles and their release in environment may be considered. The interaction of nanoparticles must be studied to understand and evaluate the risks of these nanoparticles.

2.2. Recent developments in nanotechnology

Nanotechnology glowing in every field of life including plant sciences by scientific novelties. Although the used of nanotechnology in plant sciences is at the early stage, however, it appears to have significant effects in different areas. Nanotechnology has great potential and can serve different field related to plants sciences and environment. Different nanoparticles are being used in food productions and food processing industries. They can also used be used as fertilizer as well as pesticide. In plants the same principles can be applied for a broad range of applications, particularly as nutrition supplement and growth catalysts. Nanoparticles can be labeled agrochemical or other substances as carrier agent to the plants systems for the controlled release of nutrient. Doing so, the negative effect of nanoparticles must not be neglected, such as phytotoxicity, growth inhibition etc. In this scenario, there is need to predict the environmental effect of these nanoparticles in the near future.

2.3. Plants nutrition

Both silver and zinc are very important for plant nutrition. Although silver is not any micro or macro nutrient for plant growth, but according review of literature silver can enhance plants growth and development. Silver nanoparticles (SNP) are good materials having antifungal and antibacterial activities, and used in nutriment and agriculture such as food safety, food packaging and pathogen detection (Quadros & Marr, 2010). It has great impact on plant growth and development such as germination, root-shoot ratios, seedling growth, root growth and senescence inhibition (Shah & Belozerova, 2009, Ma *et al.*, 2013). The antibacterial microbial properties of silver made it are major content in several agriculture products. Silver nanoparticles is one the most used NP in biological research due to their antimicrobial properties and do not produces any adverse effect on plants (Pandey *et al.*, 2014). Zinc is micronutrient for plants which is involved in many physiological functions. Zinc deficiency causes reduction in plant yield, stunting growth, decreasing numbers of tiller, Chlorosis and smaller leaves, increasing crop maturity periods, spikelet sterility and inferior quality of harvested products. Zinc also play most imported in cellular function in all organisms. The control and preservation of the gene expression required for the fluctuation of environmental stresses in are zinc depended. Zinc also involved in activation of enzymes that are responsible for carbohydrate metabolism, protein synthesis, controlling of Auxin productions and pollen formation (Hafeez *et al.*, 2013).

2.4. Entry of nanoparticles in plants

The cell wall of plants cell serves as protective shield and prevent external particles including nanoparticles to enter into plants cell. The pores size of plants cell wall is very small, ranging from 5 to 40nm in size so those nanoparticles which have small size then cell wall pores can enter into plants cell (Navarro *et al.*, 2008).

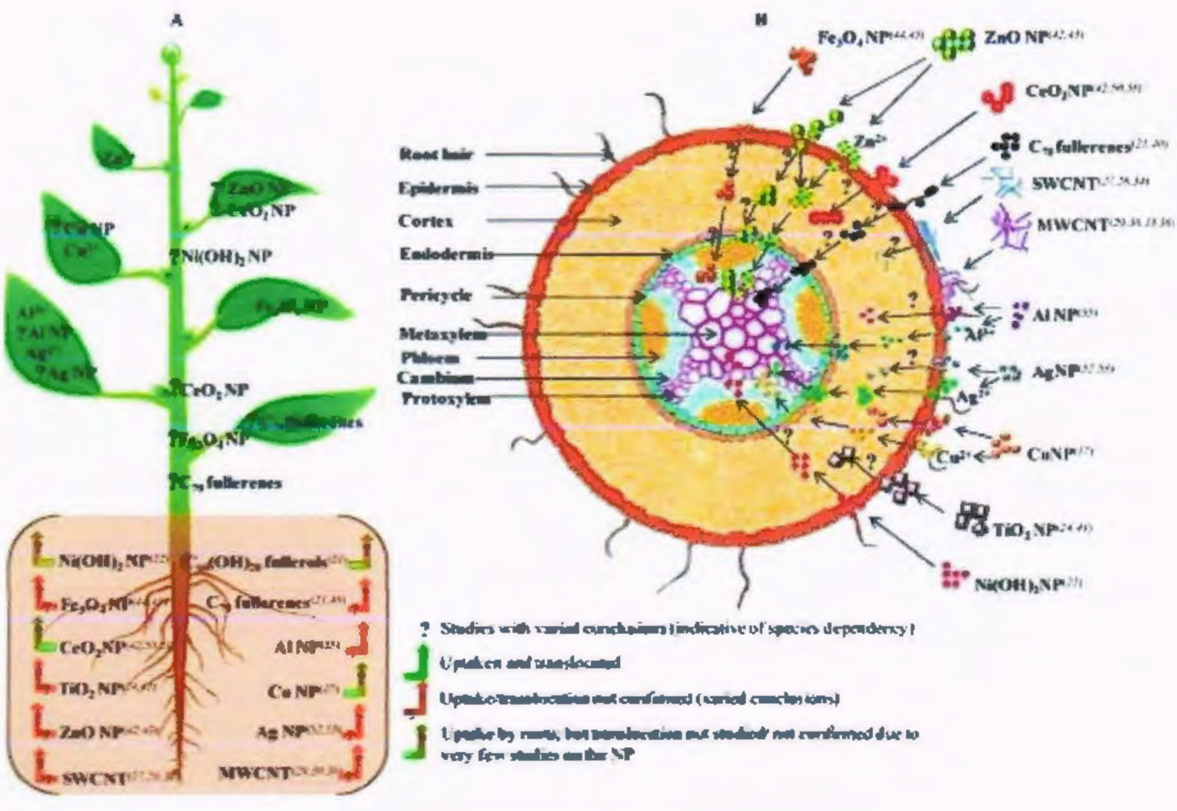


Figure 2.1: Diffusion, Transport and Bioaccumulation of nanoparticles into plant cell (Rico *et al.*, 2011)

Some time due to interaction of nanoparticles with plants the pores size become large and may also be the consecration of new cell pores due to which the uptake of nanoparticles into plants cells become increases. The other mean of nanoparticles entry to plant cell is endocytosis. Plant cell are ingested nanoparticles with help of endocytosis. The nanoparticles can interfere with metabolic process when bind with cytoplasmic organelles inside the cytoplasm (Jia *et al.*, 2005). The anther way through which nanoparticles is enter to plant body is leaves stomata from where its transfer into different tissues. The assemblage of nanoparticles in plant motivates to explore the effect of various nanoparticles on plants.

2.4.1. Nutrient pathway for the silver uptake by plant

There are two main pathways in plant for silver nutrient supply, one is mass flux pathway (MFP) and other is diffusion. Mass flux refers to the pathway that allows the movement of salutes along with the flow of their solvent (i.e. water) towards the root surface, where they are consumed. This pathway is regulated by transpiration, in transpiration water loss from the aerial parts of the plants due to which pressure produce on roots and root absorbed more water along with nutrient.

Translocation of silver, in plant body are not fully known, according to literature the accurate way of silver in root tissues is not clear known but much of it is to be reduced to its metallic state. Since silver ions have high attraction Sulfhydryl (R—S—H), Amino groups (R—NH2) and Imino group (NH2—CH—R---COOH). It is accepted that the accumulation of these ions results in their binding to or they form complexes with membrane components and possibly active site of some enzymes so changes the membrane permeability.(Koontz & Berle, 1980).

2.4.2. Nutrient Pathway for the zinc uptake by plants

Mostly zinc transport in plant trough phloem. Plants absorbed zinc in dissolved form with help of root xylem, from xylem tissues, zinc transfer to phloem and move upward. Successful transport of zinc in xylem has been prove previously especially toward wheat ear. The movement of zinc from xylem to phloem during transport toward developing wheat grain may occur in either rachis or peduncle. Xylem to phloem may also occur in the crown where roots meet the stem(Haslett *et al.*, 2001).

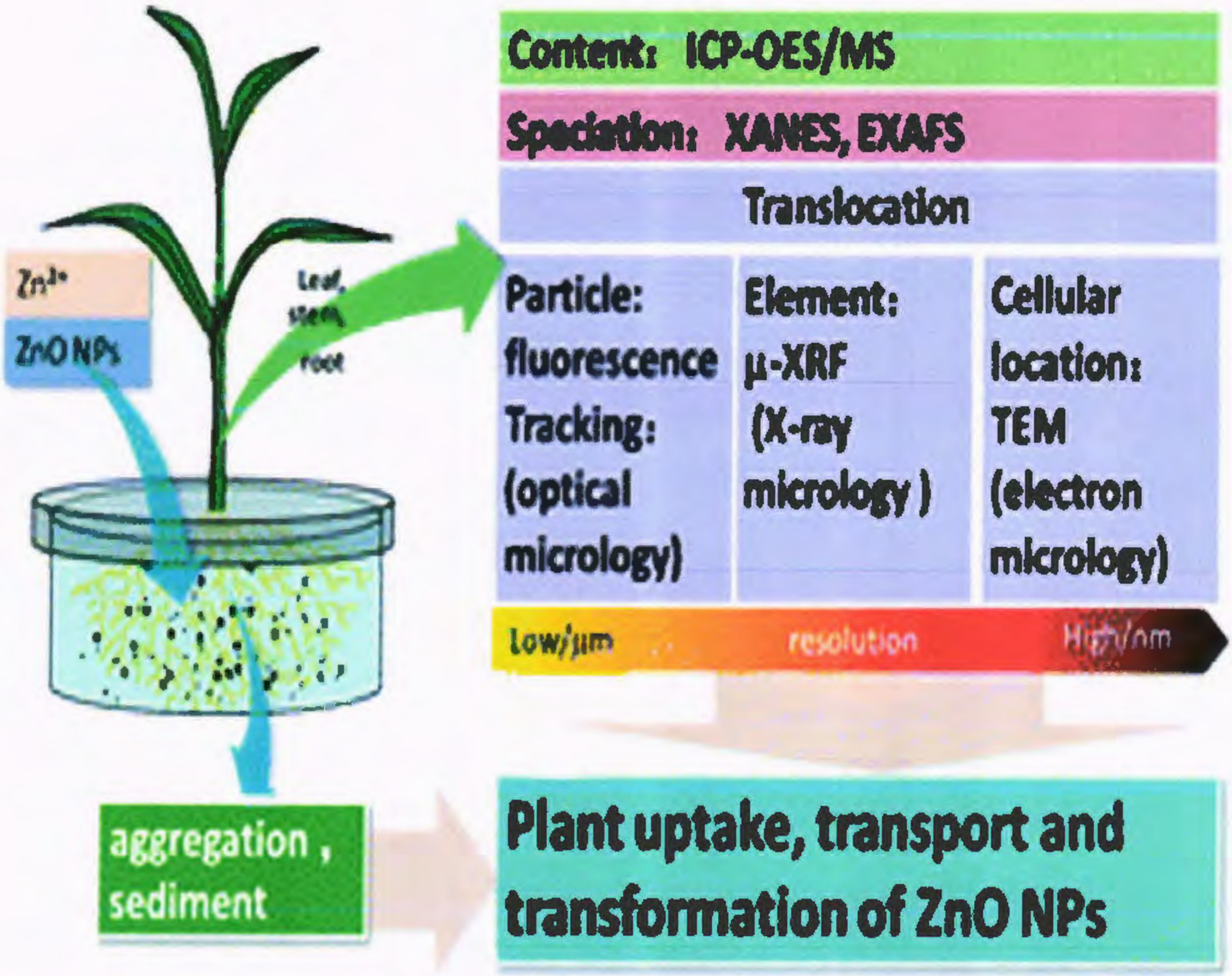


Figure 2.2: Zinc oxide nanoparticles pathway in maize plants, accumulation, speciation and uptake of ZnO NPs (Lv *et al.*, 2015).

2.5. Destiny of nanoparticles in plant sciences

In recent researcher, used nanoparticles in different area of research, to study different aspects of nanoparticles on plant growth and development. These nanoparticles can be useful and safe for growth and development and some time unsafe and show negative effect for plants. There are different studies available for nanoparticles in literature for plant development and growth (Ma *et al.*, 2013, Pandey *et al.*, 2014). Most of the studies were conducted hydroponically for short periods of growth. Applications of nanoparticles in different culture mediums respond differently.

2.6. Application of Nanoparticles in plant studies

Nanoparticles are widely used in different field of research, Therefore their interaction with surrounding environment is one of the vital problems. Nanoparticles can enter the agro ecosystems by different possible way such as water, soil and plants. Up to date different studies about nanoparticles, their application and their bioaccumulation in plants have been reported. Researchers have also concentrated on the effect and mechanisms of different nanoparticles on plants. (Zheng *et al.*, 2005)

2.7. Passage way and translocation of various nanoparticles in plants cell

Plants provide possible route for the transfer of nanoparticles to the surrounding and untimely surface way for their accumulation into the food web. Different research observations provide evidence about nanoparticles and plants response against these nanoparticles. The plants response may be negative or positive to the provide nanoparticles. Researcher also studies their mechanisms i.e. how they enter to the plant body and they affect their growth and development. As we know that plant cell wall are hard and tough and not allowed the smooth entrance of any external material as well as nanoparticles into plant cell. The entry and exit feature of wall of the cell depend on the diameter of pores present on the cell wall (Rondeau-Mouro *et al.*, 2008). Therefore nanoparticles within range could be enter through cell wall and plasma membrane and transferred to the aerial parts of the plants.

Nanoparticles might induce many morphological changes in the roots structures due to the magnification of pores and stimulation of new pores in cell wall, which ultimately enhance the absorption of nanoparticles, their aggregate or complexes. During the process of endocytosis plasma membrane folded around the nanoparticles and forms a pouch like shape due to nanoparticles internalization. Cell membrane either used insert transport carrier protein or through ions channel for the entries of nanoparticles. In the cytoplasm the nanoparticles may attached to various cytoplasmic bodies and inhibit with metabolic process at the point (Jia *et al.*, 2005). Conversely nanoparticles assemble on photosynthetic and induced foliar heating that can alter the gaseous exchanges due to stomata disturbance. Therefore change the different molecular and physiological function of the plants (Silva *et al.*, 2006). The mechanisms of translocation and influence of different nanoparticles within the plants need to be furthers investigation, to determined the whole mechanisms and their behavior in plants(Nair *et al.*, 2010).

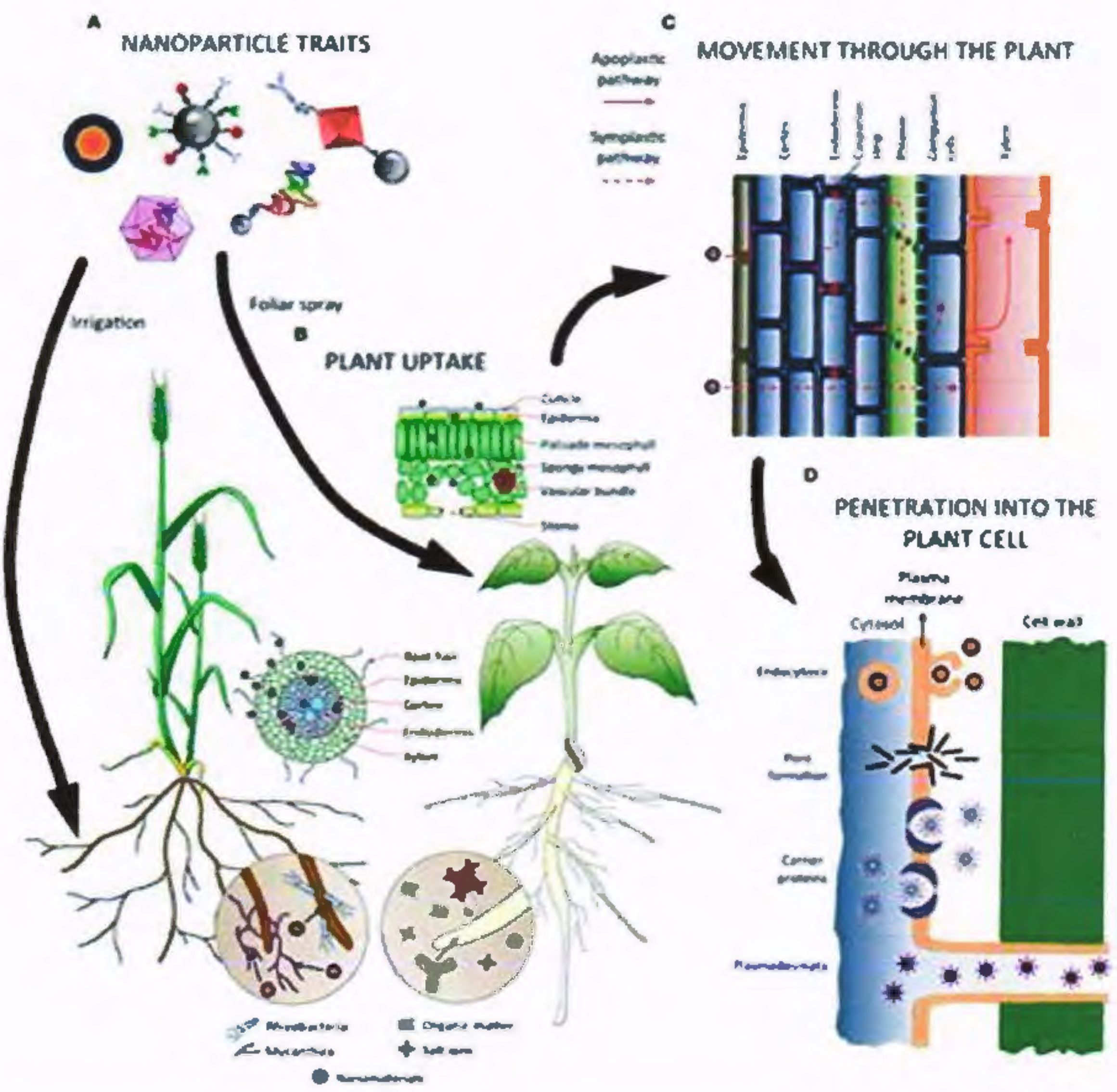


Figure 2.3: Absorptions, uptake, transport and penetration of nanoparticles in plants (Pérez-de-Luque, 2017).

2.8. Influences of various nanoparticles on plants

Scientists have find out the effect of different nanomaterials on plant growth and development. These nanoparticles have certain effect on various plants parameters that are studied. So it is very imported to understand the effect of various nanoparticles on crop plants.

2.8.1. Impact of carbon nano tubes and multi walled carbon

In advanced nanotechnology research, carbon nano tubes (CNTs) are used to deliver various bio molecules and drugs into the cell. Nanoparticles have the ability to perforated and migrated to the protoplasm of the plants tissues (Corredor *et al.*, 2009). The effects of CNTs on tomato (Lycopersicum esculentum) were studied and their impact on seed germination and growth rate was absorbed. The seed which is treated with CNTs with range of 10-40mg/L showed more growth is compared to untreated seeds. Additional studies exposed that the carbon nano tubes has ability to penetrated in thick coat of the seed and improve water accumulation in seed, which is in turn effect on seed sprouting general growth of tomato seedling (Khodakovskaya *et al.*, 2009).

CNTs (functionalized and non-functionalized) were also applied to six different types of plants (onion, tomato, cabbage, carrot, cucumber and lettuce) to study the effects on roots elongation. These plants species were normally used phytotoxicity test. The results showed that the non-functionalized CNTs had more effects on roots lengths than functionalized nano tubes. Observation on non-functionalized nano tubes show that they enhance the roots elongation in onion and cucumber but inhibited in tomato roots lengths. Functionalized nano tubes found to slow down roots elongation in lettuce plants. None of the nano tubes affected the cabbage and carrot plants species. Microscopic images showed that the existence of nano tubes on root surfaces while uptake of nano tubes was not observed (Cañas et al., 2008).

2.8.2. Effect of magnetic nanoparticles

The most imported properties of magnetic nanoparticles are that, it is used for the delivery at target site in plants and other organisms. The uptake of magnetic nanoparticles and their translocation inside pumpkin plants in the range of <50 have been observed(Corredor *et al.*, 2009). Iron oxide coated with tetramethylammonium hydroxide (TMA-OH) was used as stabilizing agents in maize plants. At low concentration of these nanoparticles enhance the "chlorophyll a" contents while at higher concentration reduce the "chlorophyll a" contents. The maize seeds were exposed to electromagnetic field in the presence of magnetic fluid, the assimilatory pigment were reported decrease as the concentration of magnetic fluid solution increases. The plants tissues absorbed the magnetic field energy by incorporate magnetic nanoparticles and influenced the redox reactions. Redox reactions activated photosynthesis process resulting in increased level of nucleic acid.

The magnetic nanoparticles might also cause some magnetic effects of enzymatic activities of plants that took part in photosynthetic and development process. So that is way to it is required to reduce the concentration range of Ferro fluid for further used in plants for better yield of crops with improved photosynthetic pigment level(Racuciu & Creanga, 2007). In another experiment three types of treatment were used, Fe nanoparticles, Fe nanoparticles associated with organic fertilizer and with humic acid were applied to transfer and photosynthates to the leaves. Result they report that iron oxide nanoparticles act as catalyst for iron transfer to the leaves of peanut. They found increased iron contents in leaves about 218, 207 and 206 mg/kg respectively (Liu *et al.*, 2005). Fe3O4 were applied to pumpkin seedling and result showed that the accumulation of nanoparticles by plants roots, stems and leaves (Zhu *et al.*, 2008). Iron oxide nanoparticles were applied to soybeans with different concentration. Result showed that iron oxide nanoparticles with the concentration level of 0.75g/L were increased leaves and pod dry biomass. The maximum grain products gain through concentration of 0.5g/L which increase 48% products over control (Sheykhbaglou *et al.*, 2010)

2.8.3. Effect of titanium dioxide nanoparticles

The effect TiO2 were studied on spinach seed germination and growth and found that TiO2 NPs enhance light absorbance and enhance the activities of Rubisco Activase. So TiO2 NPs act as a photo catalyst which increased spinach growth. In antanase phase TiO2 NPs observed that it is increases growth due to improved nitrogen metabolism, more inorganic nitrogen converted into organic nitrogen, more organic nitrogen increases the fresh and dry biomasses of plants from 91% up to 99% as compared to control. Total nitrogen also increases chlorophyll and protein contents up to 23.35% (Yang, 2007).

The effect of nano- TiO_2 and non-nano- TiO_2 were also studied on aged spinach seed germination and growth. The effect of nano- TiO_2 was reported that it is increases chlorophyll and protein contents in spinach and also increased the antioxidant stress due to lower accumulation superoxide that ultimately helped the spinach chloroplast to release more oxygen in the presence of UV-B radiation (Zheng *et al.*, 2005).

Another studied of TiO_2 NPs were conducted on chickpea plants and found that low concentration of TiO_2 NPs were useful for cell membrane. The positive effect was found on chickpea cell when they were exposed cold stress. The tested concentration level did not induce any morphological changes, perhaps due to their low concentration levels. TiO_2 NPs at 5mg/kg concentration levels were studied to reduced cold-induced damage in sensitive and resistant chickpea genotype. Such effect raise the question about the possible mechanisms, how they effects. The possible answer is that the chickpea seed activated some defensive mechanism in seedling after absorption of UV NPs. So supporting the plants in cold stress. These results are very interesting to more practicing in case of environmental stressed conditions. The new investigation possibly would cover the way for the use of the nanoparticles especially for the increases of cold tolerance in major crops (Mohammadi *et al.*, 2013).

Arabidopsis thaliana were tested for uptake of sucrose coating of 43% TiO_2 with size less than 5nm. Result show that small nanoparticles enter to cell and acquire accumulated in distinct sub cellular layer (Kurepa *et al.*, 2010). In same way the application of 40mg/kg of TiO_2 NPs was reported to enhance the average germination time about 31.8% as compared to control. Another research contacted by wheat plants, TiO_2 NPs size less than 140nm. Wheat roots were studied. The nanoparticles size less than 36nm can easily transported to the leaves of wheat plant, but their concentration was below from the detection levels. By the accumulation of TiO_2 NPs up to 14nm and 22nm the roots/shoot elongation were observed, but no changes were observed in seed germination, vegetative development, photosynthesis or redox balanced (Larue *et al.*, 2012).

The effect of TiO_2 nano substances on plants growth has also been studied. Result show that positive effect occurs in photosynthesis and plants growth due to applied TiO_2 nanoparticles. But their mechanism was still ambiguous. Basically the capturing of light in chloroplast and light harvesting complex II was supposed to be stimulated by TiO_2 nanoparticles, so enhancing the transformation of light energy to electronic energy, evolution of oxygen and water photolysis (Ze *et al.*, 2011).

2.8.4. Effects of aluminum nanoparticles

Aluminum nanoparticles (Al NPs) have been used for different applications, so there are more chance to release in environment and their relation with higher plants. Different studied have been conducted by using pure aluminum nanoparticles, about 13nm aluminum nanoparticles were used on different plants species such as corn (*Zea mays*), cucumber (*Cucumis sativus*), cabbage (*Brassica oleracea*), soybean (*Glycine max*) and carrot (*Daucus carota*). Roots inhibition was reported in all these plants by using aluminum nanoparticles (Yang & Watts, 2005).

Aluminum nanoparticles with suitable surface modification were studied to reduce the photo toxicity effect. The Al_2O_3 and coated Al_2O_3 with carboxylate ligands (100nm) showed no inhibitive effect on the red bean (*Phaseolus vulgaris*) and rye grass (*Lolium perrene*) growth. About 2.5 fold increased were seen with concentrated aluminum nanoparticles over control in rye grass but there were no uptake reported in red bean (Doshi *et al.*, 2008).

Phosphorus loaded aluminum oxide nanoparticles as used as a source to release bound phosphorus in hydroponic. In *Brassica napus* phosphorus uptake was studied which was increase plants growth. It was found that about 8fold increased at low concentration of phosphorus over control one (Santner *et al.*, 2012).

2.8.5. Effect of zinc oxide nanoparticles

Zinc oxide nanoparticles were used on zucchini seeds in hydroponic solution. Results were shown that there were no inhibitory effects on seed sprouting and root growth. Similarly ZnO nanoparticles were also applied on rye grass and corn 35 and 15—25nm size. Result shown negative effect seeds germination were inhibited (Lin & Xing, 2007). ZnO nanoparticles were also used to maintain more soluble and diffusible source of zinc fertilizer to overcame the zinc deficiency which is of the major issues prevent agriculture productivity in alkaline calcareous soil (Milani *et al.*, 2010).

In another research work was conducted by rye grass plants treated with in nutrient solution with zinc oxide nanoparticles and result showed harmful effects at higher concentration. Zn ions showed more harmful effect than zinc oxide. Zinc oxide uptake image were verified by SEM and proved to damage the epidermal and cortical cells of plants. Nanoparticles aggregate were formed that block the pores and channels, so there is need to do further research on this studied minimize the risk of photo toxicity assessments. In another research work the uptake of Zn and ZnO nanoparticles were studied. The result of this studied were showed that at different concentration. The movement of Zn by corn range from 69 to 409mg/kg in roots and 100 to 350mg/kg in shoots, when grown in soil treated with ZnO nanoparticles from 100 to 800mg/kg for one month exposure time. The entries of ZnO nanoparticles is verified by Confocal microscope image the zinc oxide nanoparticles were enter to roots epidermis and cortex through apoplastic pathway. In the xylem vessels nanoparticles aggregates of nanoparticles the epidermis through symplastic pathway (Zhao *et al.*, 2012).

The impact of ZnO nanoparticles on, mung (*Vigna radiate*) and gram (*Cicer arietinum*) seedling were studied. The plant agar method was used for this experiment to avoid settling of nanoparticles in test pots. Various concentration of zinc oxide nanoparticles were used into agar medium and their impact were studied by mean of roots and shoots growth parameters in seedlings. The uptake and transport of zinc oxide nanoparticles in roots were verified by SEM (Mahajan *et al.*, 2011)

2.8.6. Effects of copper nanoparticles

A research work was conducted on mung bean and wheat plants to determine the effect of copper nanoparticles by using plant agar culture media. Results showed that mung bean seedling was inhibited by copper nanoparticles while in wheat seedling there is no inhibition was seen. Mung bean was found more sensitive to copper (Cu) nanoparticles than wheat. The presence of copper nanoparticles which is translocated across the plasma membrane was conformed through TEM images. In another study, when zucchini plants exposed to copper nanoparticles it was found that roots length were reduced (Stampoulis *et al.*, 2009).

Research study on lettuce plants germination showed that copper nanoparticles increased shoots to roots ratio as compared to control one. (Shah & Belozerova, 2009).

2.8.7. Effect of silver nanoparticles

Research study were conducted on silver nanoparticles and silver nitrate bulk form on mustard plant (*Brassica juncea var Varuna*) to assess the toxicity of these particles. Result showed that silver nanoparticles enhance the roots and shoots length and also increase the protein and chlorophyll contents as compared to control one while silver nitrate reduce roots and shoots length and also reduce chlorophyll and protein contents (Pandey *et al.*, 2014). Another experiment was conducted on zucchini plants in hydroponic culture. Plants biomass and transpiration were studied and reported that the exposed plants to silver nanoparticles plants biomass and transpiration were decreased and plant growth was found to be prolonged (Nair *et al.*, 2010). Different flora and fauna react in different way into different nanoparticles. So before experimentation it is very imported to estimate their useful concentration and their harmful concentration that is considered to be safe or harmful to reduce the risk of ecotoxicity.

Chapter. 03

MATERIALS AND METHODS

This section describes the experimental structure of the present study. The research study is divided into two main sections. In the first section synthesis and characterizations of Ag and ZnO nanoparticles were carried out, and the second section the nanoparticles were applied to wheat plant. The whole experiment was conducted in SA-CIRBS IIUI. First examination focused on the plant growth and second purposes focused on analysis of various biochemical parameters.

3.1. Synthesis of zinc oxide nanoparticles

Zinc oxide nanoparticles were prepared by co-precipitation method. In this method zinc nitrate (ZnNO3) and potassium hydroxide (KOH) were used as a raw material. First of all aqueous solution of 0.2M of ZnNO3 and the solution 0.4M of KOH were prepared in double distilled water. Then KOH solution was added drops wise to ZnNO3 solution with continues stirring at room temperature. After some time a white suspension were formed. The white suspension was centrifuged at 10000rpm for 20minutes. The supernatant was discarded and the pellet was washed four times with distilled water. The product was calcined at 500°C in air atmosphere for 60minutes, the white zinc oxide nanoparticles powder prepared (Ghorbani *et al.*, 2015, Nadhman *et al.*, 2016). The ZnO NPs were suspended in deionized water (DI) and stock solution were prepared.

3.2. Synthesis of silver nanoparticles

Syntheses of silver nanoparticles (Ag NPs) were also carried by modified co-precipitation method. For synthesis of silver nanoparticles, silver nitrate ($AgNO_3$) and sodium hydroxide (NaOH) were used as precursor molecules. The solution of $AgNO_3$ 0.2M and the solution of NaOH 0.4M were prepared in de ionized water. The solution of NaOH was added to the solution of $AgNO_3$ drops by drop at room temperature. The $AgNO_3$ solution was stirring continuously. After some instant, white suspensions were formed. The white suspensions were centrifuged at 10000rpm for 20minutes. After centrifugation two layers were formed, upper supernatant was discarded and lower pellet were washed four timed with distilled water. Then the product was calcined at 500°C in air atmosphere for 1 hour. After that gray color silver nanoparticles powder were prepared (Ghorbani *et al.*, 2015, Nadhman *et al.*, 2016).

3.3. Characterization of silver and zinc oxide nanoparticles

After preparation of Ag NPs and ZnO NPs by co-precipitation method. The particles size and shape of Ag NPs and ZnO NPs were study through Scanning Electron Microscopy (SEM). Crystalline structure of Ag NPs and ZnO NPs were find through X-Ray Diffraction (XRD). For the chemical characterization of the particles Energy-Dispersive X-ray Spectroscopy (EDS) were done.

3.3.1. Morphology of Ag Ps and ZnO NPs (SEM)

Scanning Electron Microscopy very sensitive and strong tool to study the morphology of different types of nanoparticles. In this technique a high accelerated electron bean is generated in a vacuum by very high voltage. Various signals are produced when a beam of electron strike the sample. The backscattered electrons and signal are collected by electron collector and these are used for showing sample morphology and image is formed by magnetic lenses. In the present observation the morphology of Ag NPs and ZnO NPs were study by using JEOL JSM-6460 SEM.

3.3.2. Characterizations of Ag NPs and ZnO NPs (XRD)

X-Ray diffraction is analytical technique used rapidly for the phase determination of crystalline material and can provide very sensitive and authentic data. It is commonly used for the verification of crystal structure and atomic spacing. In this technique the beam of X-ray is produced and directed toward the sample. The interaction of the incident X-ray with sample generated diffracted rays at different angles. The diffracted rays are collected giving a high peak, which show the crystallinity of the sample. In our study Shimadzo 6000 X-Ray diffracto-meter was used to determine the crystallite size and crystal structure of Ag NPs and ZnO NPs.

3.4. Preparation of stock solutions

After synthesis and characterizations silver and zinc oxide nanoparticles by co-precipitation method it is suspended in double distilled water and one stock solution silver nanoparticles and one stock solution of zinc oxide was prepared. Each stock solution were further dilute with double distilled water and 37ppm, 75ppm, 150ppm and 300ppm concentration solutions were prepared.

3.5. Insertion of different concentrations of solutions to petri plate

The prepared solutions of different concentrations were poured into different petri plate. The petri plates compose by wheat seeds. Each petri plates have ten numbers of seeds. The petri plates were sealed by parafilm tape. All the petri plates were kept in plants growth chamber for fifteen days of growth. Plants growth chamber provide optimum conditions for wheat growth and developments.

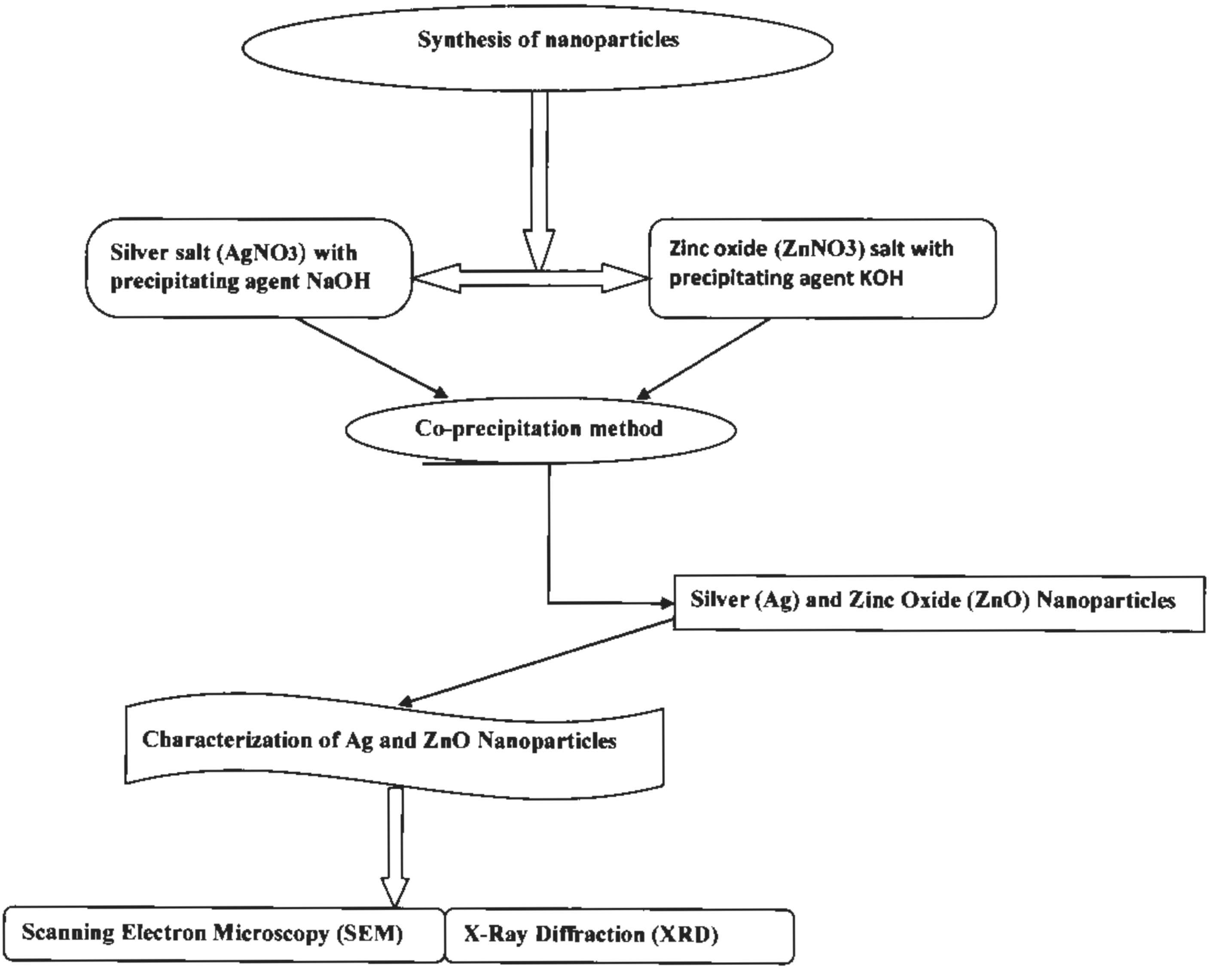


Figure 3.1: Flow charts for Ag and ZnO preparation and characterization

3.6. Seed treatment

Seeds of wheat (*Triticum aestivum*) were selected for the study to test the effect of ZnO and Ag nanoparticles on root shoot length, protein contents, lipids degradation, effect on chlorophyll and effect on proline. Seeds of *Triticum aestivum* were obtained from National Agriculture Research Centre Islamabad. The seeds were washed with distilled water and then sterilized with 5% sodium hypochlorite. After words, seeds were further thoroughly washed with distilled water. The sterilized washed seeds were immersed in distilled water for 24 hours in dark. Then the seeds were transferred to petri plate containing filter paper. The prepared 37ppm, 75ppm, 150ppm and 300ppm concentration of ZnO and Ag solutions were introduce to petri plate containing wheat seeds. After this the petri plate containing wheat seeds with different concentration of nanoparticles were kept in plants growth chamber at temperature 28°C (Model: BJPX-L500) for germination and growth.

3.7. Experimental set up

The experimental set up were performed with three replicate to examine the result of different concentration of silver and zinc oxide nanoparticles on root/shoot length, protein, lipids, chlorophyll and proline. Four different concentrations of 37ppm, 75ppm, 150ppm and 300ppm prepared and also one control without any concentration of nanoparticles prepared. After sterilization of seed about ten seed per petri plate were selected and exposed to prepared different concentration nanoparticles. Distilled water was used in the control treatment instead of Ag and ZnO nanoparticles concentrations.

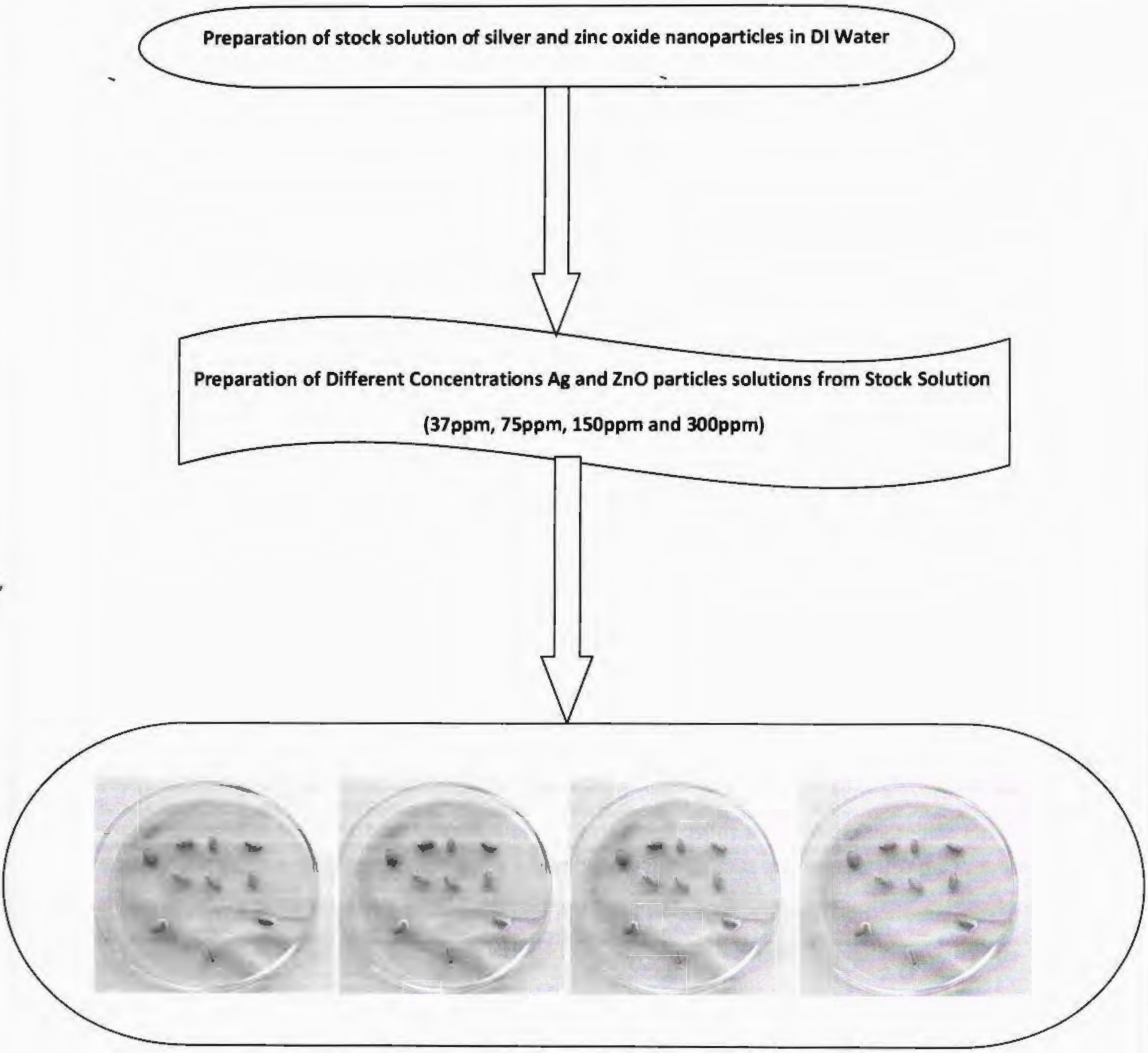


Figure 3.2: Flow sheet for Ag and ZnO solutions preparation and application to *Triticum aestivum* seeds

and the pellets were washed with 80% acetone because the pellets were also consisting by trace of chlorophyll and the chlorophyll was dissolved in the acetone. So chlorophyll was removed with acetone. The proteins were further washed with distilled water. The obtain protein was dissolved in 1ml of 0.1M NaOH and read at 595nm in spectrophotometer (PG T80+ UV/VIS-Spectrometer), the Bovine Serum Albumin (BSA) keep as a standard (Bradford, 1976).

3.8.3. Chlorophyll estimation

Chlorophyll is organic molecules and very imported pigments for glucose synthesis. Chlorophyll harvested light energy and converted into chemical energy for further uses (Yakar & Bilge, 1987). There are different kinds of chlorophyll. The most common is chlorophyll a and chlorophyll b occurring in higher plants and algae. Chlorophyll a is primary photosynthetic pigment. Chlorophyll a trap light energy and transfer to a chemical acceptor (İnanç, 2011).

The chlorophyll was extracted with 80% acetone. Eight treated (silver and zinc oxide treated plant) and one untreated wheat plants leaves were selected and isolated from plants. Fresh leaves of wheat leaves were weighted. About 0.5g fresh leaves were homogenized in 80% acetone with help of pestle and mortar. After the homogenization the contents were centrifuged at 1000rpm for 15minutes at 4C°. The upper layers supernatant were consists by chlorophyll so the pellet were discarded and the supernatant were transfer to fresh test tube. The green supernatants were further analysis by spectroscopic analysis. The spectroscopic analysis were carried out under UV/VIS spectrophotometer (PG T80+ UV/VIS Spectrometer) at wave length 700nm and 400nm against 80% acetone blank (Sumanta *et al.*, 2014).

3.8.4. Lipids analysis

Lipids are class of naturally occurring organic molecules. Lipid included by waxes, fats, sterol fats soluble vitamins, manoglyceride, triglyceride and phospholipids etc. the main function of lipid is storing large amount of energy, singling and structure components of plasma membranes (Fahy *et al.*, 2009, Subramaniam *et al.*, 2011).

Lipids of eight treated and one untreated *Triticum aestivum* roots were analysis by Fourier Transforms Infrared (FT-IR), model Shimadzu Fourier Transform Infrared IR Tracer 100 Spectrophotometer. Roots were cut from four Ag NPs treated wheat plants, four ZnO NPs treated wheat plants and one untreated wheat plants. The cut roots were air dried. The dried roots were mixed with dry potassium bromide (KBr). About 5mg roots were mixed with 245mg KBr with help of pestle and mortar. The samples were subjected in a hydraulic presser and press with pressure of about 5×10^6Pa. After pressing the simple about 13mm diameter and 1mm thick clear soft disc (tablet like) were formed. The discs were subjected into Fourier Transforms Infrared device. The IR spectra region 3500—500 were recorded at room temperature on FT-IR spectrophotometer (Mohan, 2004)

3.8.5. Proline estimation

Proline is a type of proteinogenic amino acid. The main function of proline is primary metabolism during stress conditions. Plants species accumulated proline in the responses of many environmental stress such as drought, high light, high salinity, heavy metals and others abiotic stresses (Szabados & Savoure, 2010). Proline acts as an active asmolytes, a metal chelater, singling molecules and an antioxidant (Yaish, 2015). Proline is only distinctive amino acid where the α-amino group is present as a secondary amine and plants that is present in stressful conditions have high amount of proline accumulations (Verslues & Sharma, 2010).

We were examined proline in wheat plants roots and shoots described by Bates et al. Total nine samples of *Triticum aestivum* were selected. Four samples were Ag NPs treated, four were ZnO NPs treated (37ppm, 75ppm, 150ppm and 300ppm) and one is untreated (0ppm) wheat plant. First of all the samples of wheat roots and shoots were frozen with liquid nitrogen and frozen samples were homogenized with 3% aqueous sulphosalicylic acid (0.02g/1ml) and then centrifuged at12000rpm for 10minutes. After centrifugation the supernatants were transfer to fresh test tube and pellets were discarded. After that 1ml of acid ninhydrin and 1ml of glacial acidic acid was added to the supernatants in test tube at 100C° in water bath. Acid-ninhydrin was formed in glacial acidic acid and 6M phosphoric acid with 2:1. After one hour the reactions were terminated in ice bath. 4ml of toluene was added to the reaction mixtures and mixed continuously and then left at room temperature for 35 minutes. After words, the optical density was measured by UV/VIS spectrophotometer model PG T80+ UV/VIS Spectrometer at 520nm for the upper pinkish layer containing proline. Toluene was used as a blank while D-proline was used as a standard (Bates *et al.*, 1973).

3.9. Statistical analysis

In all experiment, each treatment was conducted with three replicates. The statistical analysis of treated plants experimental values was compared with controls one. For data calculation and statistical analysis office excel (Microsoft excel 2007) and origin software (Origin Pro 8. Ink) were used. Data were presented as with standard error.

Table 3.1: nanoparticles, characterization and parameters that were studied.

Nanoparticles	**Characterizations**	**Concentrations Level**	**Parameters**
Silver and Zinc Oxide	SEM, XRD	37ppm, 75ppm, 150ppm, 300ppm	Root/shoot growth, chlorophyll, protein, lipids and proline analysis

Chapter 04

RESULTS AND DISCUSSION

4.1. Silver

Silver is one of the most imported element for all organisms. Silver nanoparticles impacted on metabolisms, reproduction and respiration of organisms (Lok *et al.*, 2007). Silver nanoparticles delay the maintenance periods of leaves from 2 to 21 days. Silver also delaying the abscission in plants (Labraña & Araus, 1991). Silver nanoparticles have the capability to enhance the roots and shoot length and also increase the chlorophyll and protein contents in *Brassica juncea L* plants (Pandey *et al.*, 2014).

4.2. Zinc oxide

Zinc is one of the imported elements for the growth of plants, animal and human being. It is a basic entity for crops nutrition. Zinc is one of the part of different enzymatic activities, metabolic process and oxidation reduction process (Hafeez *et al.*, 2013). ZnO nanoparticles are imported for plants growth and development. Usually lower concentration of ZnO nanoparticles positive effected on plants growth and development but higher concentration may negative effected on plants growth and development. The impacts of zinc oxide nanoparticles on seed germination depends on concentration of nanoparticles and plant species (Laware & Raskar, 2014)

4.3. Characterization of Ag and ZnO Nanoparticles

To observe the morphology of Ag and ZnO NPs Scanning Electron Microscopy (SEM) was used. Images obtained from SEM confirmed the presence of sponge, porous and spherical structure of ZnO NPs, while Ag NPs look like bead like spherical in structure. Figure 4a and 4b shows the ZnO NPs and Ag NPs taken by SEM (JEOL JSM-6460 SEM) at 60,000X magnification.

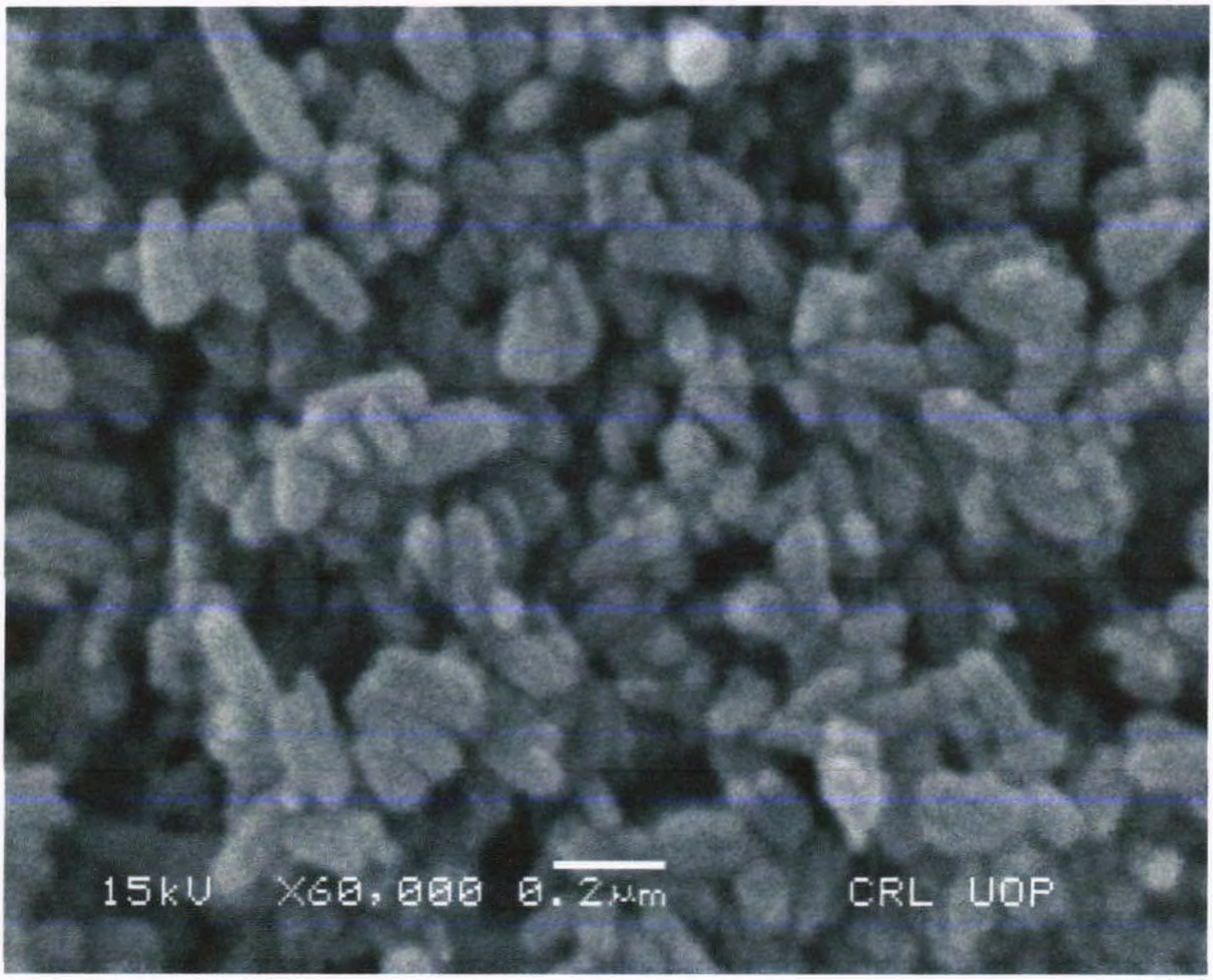


Figure 4 (a): Scanning Electron Microscopy image of ZnO NPs at X 60,000

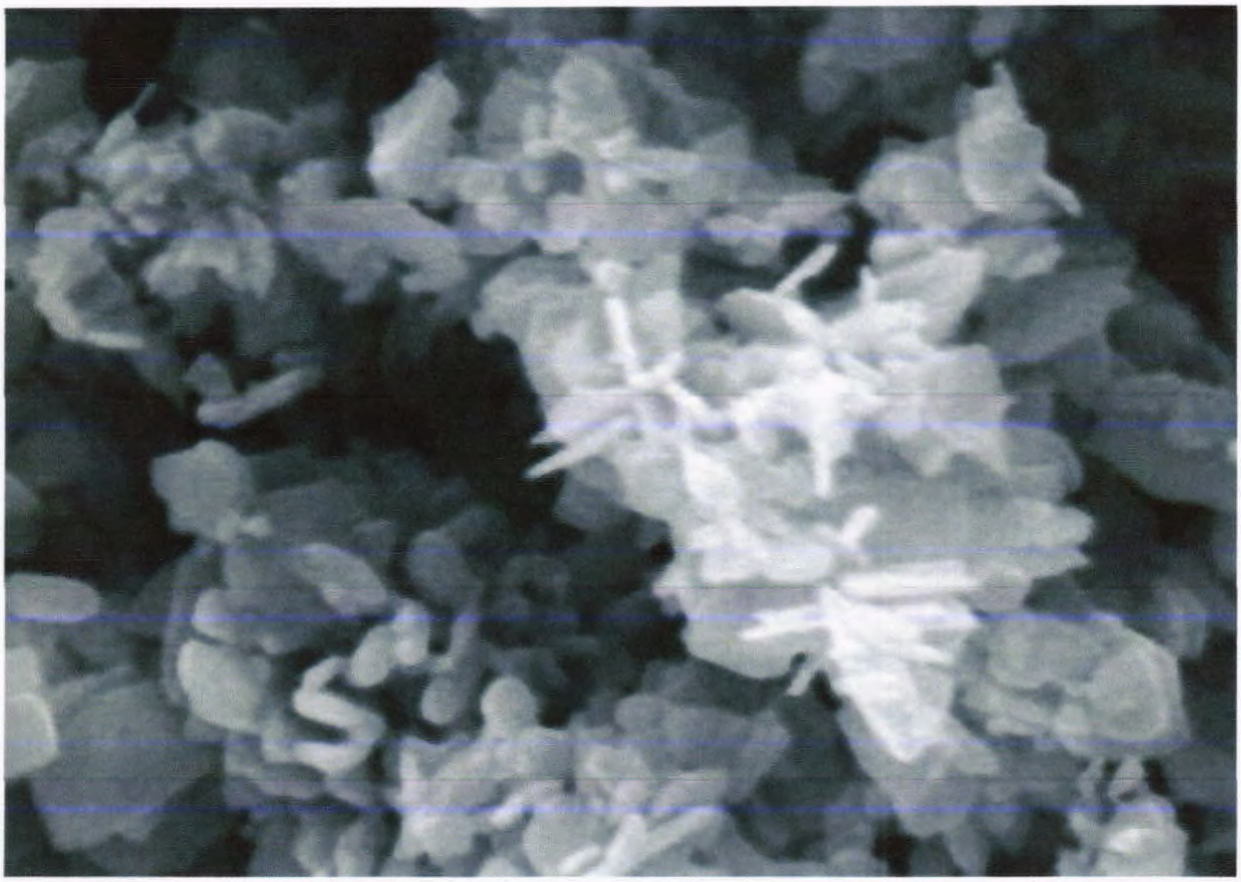


Figure 4 (b) Scanning Electron Microscopy image of Ag NPs at X 60,000

4.4. X Ray diffraction of Ag and ZnO Nanoparticles

The crystalline structure, the crystalline size and phase compositions of Ag and ZnO NPs synthesized by co-precipitations method were determined by XRD. Figure **4c** and **4d** indicate that Ag and ZnO NPs were crystalline and no amorphous phase was seen. The Ag NPs used in this study had the size of 20 to 40nm while ZnO NPs had the size of 15 to 30 nm. The Scherer formula was used to calculate the crystalline size of Ag and ZnO nanoparticles.

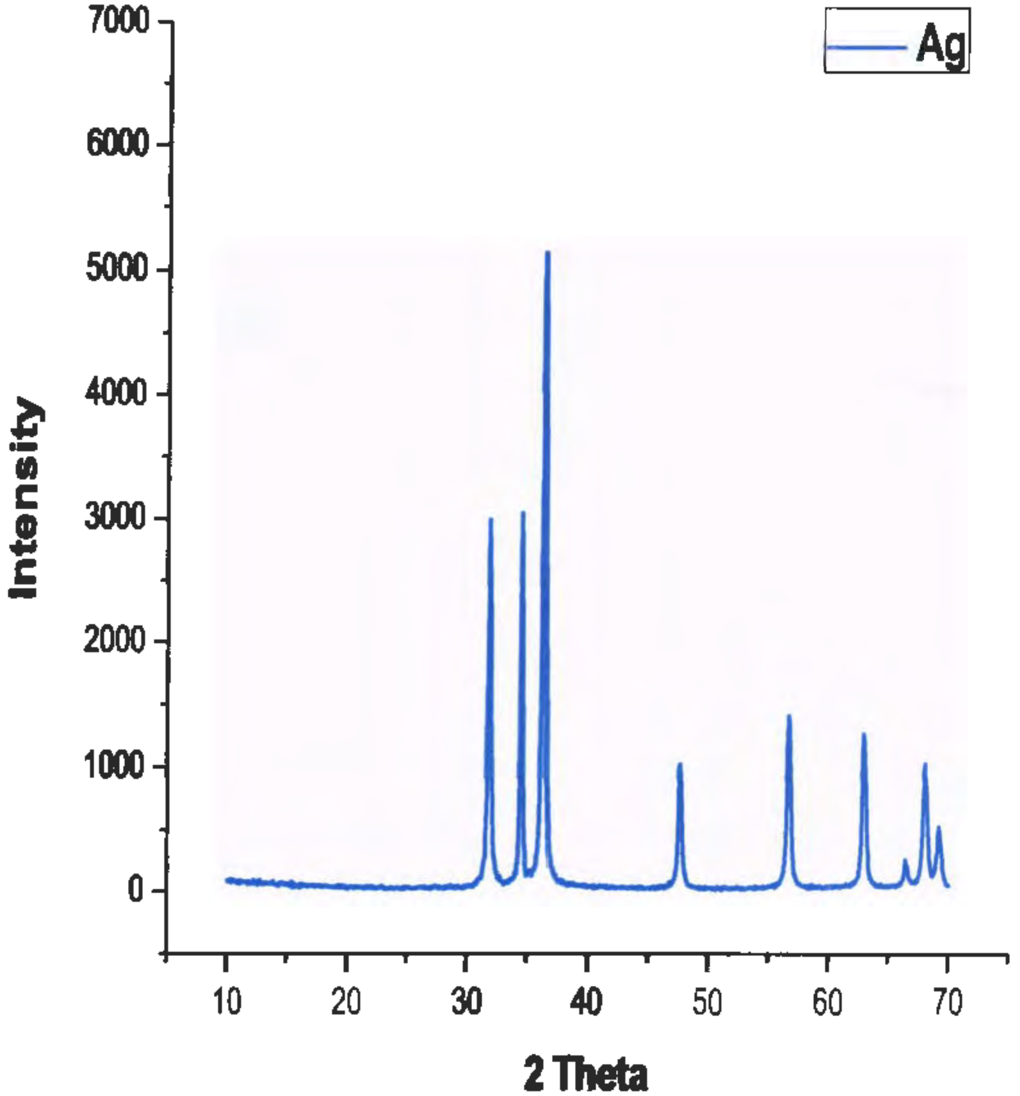


Figure 4(c): XRD pattern of Ag Nanoparticles

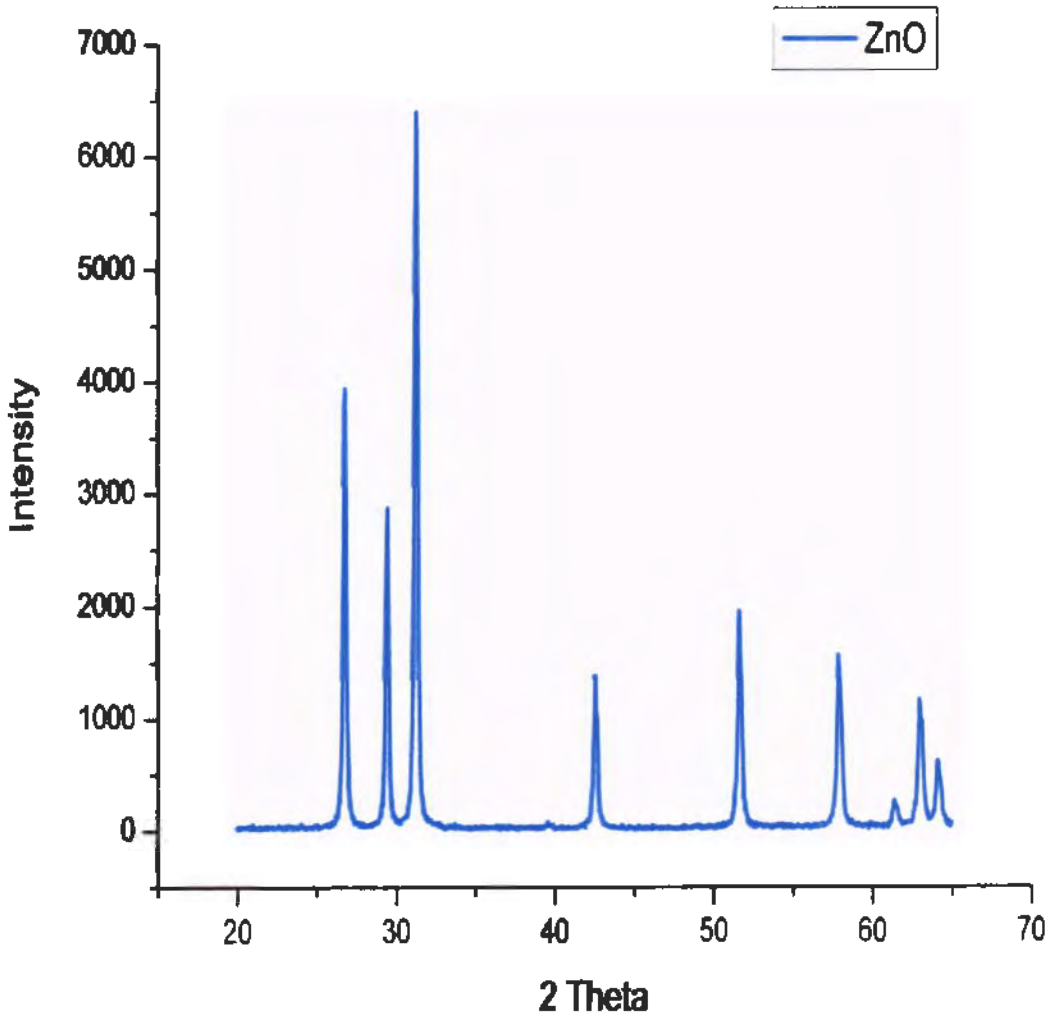


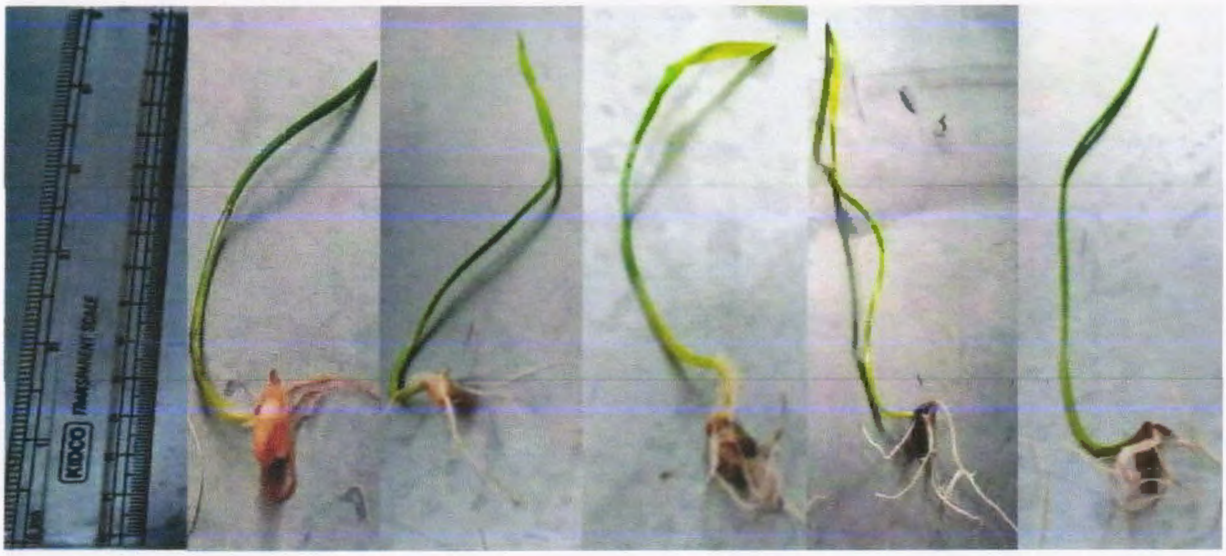
Figure 4(d): XRD pattern of ZnO Nanoparticles

4.5. Roots and shoot response to Silver nanoparticles

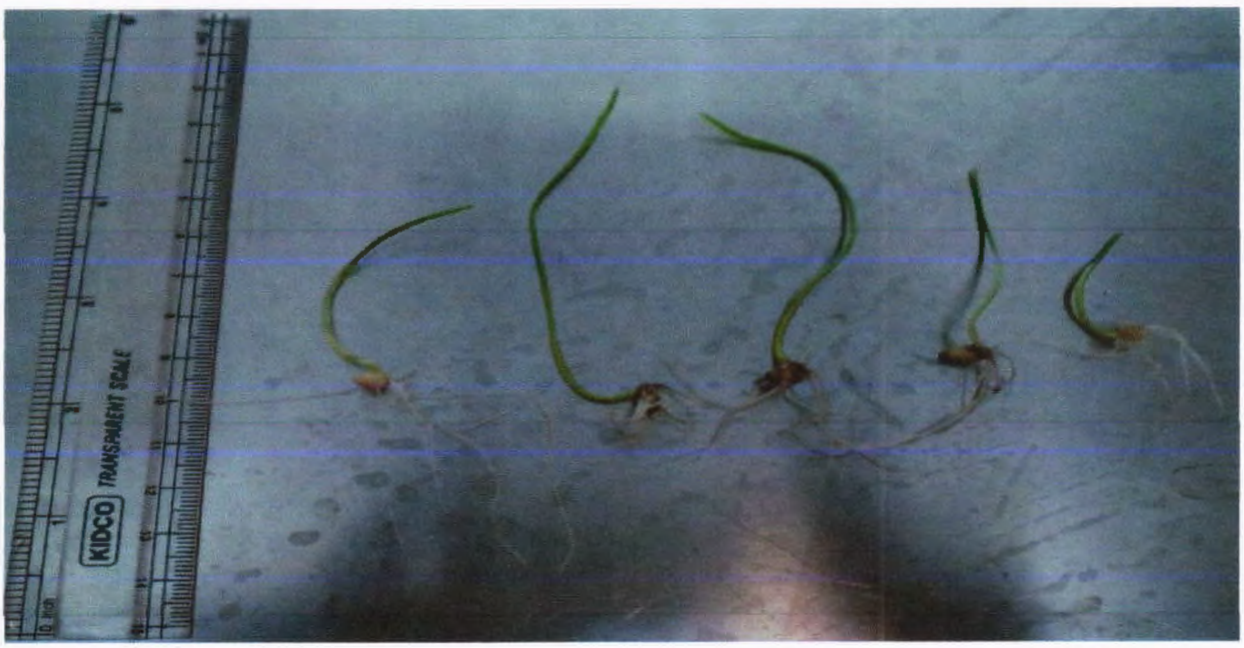
From our research study we reported that at all concentration of silver nanoparticles the roots and shoot growth were enhances, i.e. silver nanoparticles had positive effect on *Triticum aestivum* roots and shoot. We reported the significant growth in roots and shoot all silver nanoparticles treated wheat plants. Wheat treated with 37ppm, 75ppm, 150ppm and 300ppm silver nanoparticles showed longer roots and shoot growth as compared to untreated (0ppm). At 300ppm concentration of Ag NPs treated plant shoot and roots increases by 35% and 23% respectively as compared to control one. At 150ppm, 75ppm and 37ppm concentrations treated plants shoot were increased by 23%, 20% and 17% while roots length were increased by 21%, 19% and 17% respectively as compared with control one. (Figure 4.1).

4.6. Roots and shoot response to Zinc Oxide nanoparticles

In our research study we reported that lower concentration of ZnO NPs positive effected on roots and shoot growth and enhance the length of wheat roots and shoot. But higher concentration negative effected on wheat roots and shoot. The length of roots and shoot length diminish as compared to control one. As shown in figure 4.2 that at 37ppm concentration treated wheat shoot length increased 10.7% while roots length increased by 8.3% as compared to control one. Similarly at 75ppm shoot and roots length increased by 7.25% and 3%, respectively, as compared to the control. Higher concentration of zinc oxide nanoparticles (300ppm and 150ppm) were negatively affected and decreased was reported in plants roots and shoot. At 300ppm *Triticum aestivum* shoot length were declined by 5.7% and roots length were decreased by 9.2%.while at 150ppm concentration of ZnO NPs treated wheat plants shoot and roots length were decreased by 2% and 3.5% respectively as compared with control one wheat plant.



Effect of different concentrations Ag NPs on wheat plants *(Triticum aestivum)* from left to right indicated the 0ppm, 37ppm, 75ppm, 150ppm and 300ppm of Ag NPs



Effect of different concentrations ZnO NPs on wheat plants *(Triticum aestivum)* from left to right indicated the 0ppm, 37ppm, 75ppm, 150ppm and 300ppm of Z nO NPs

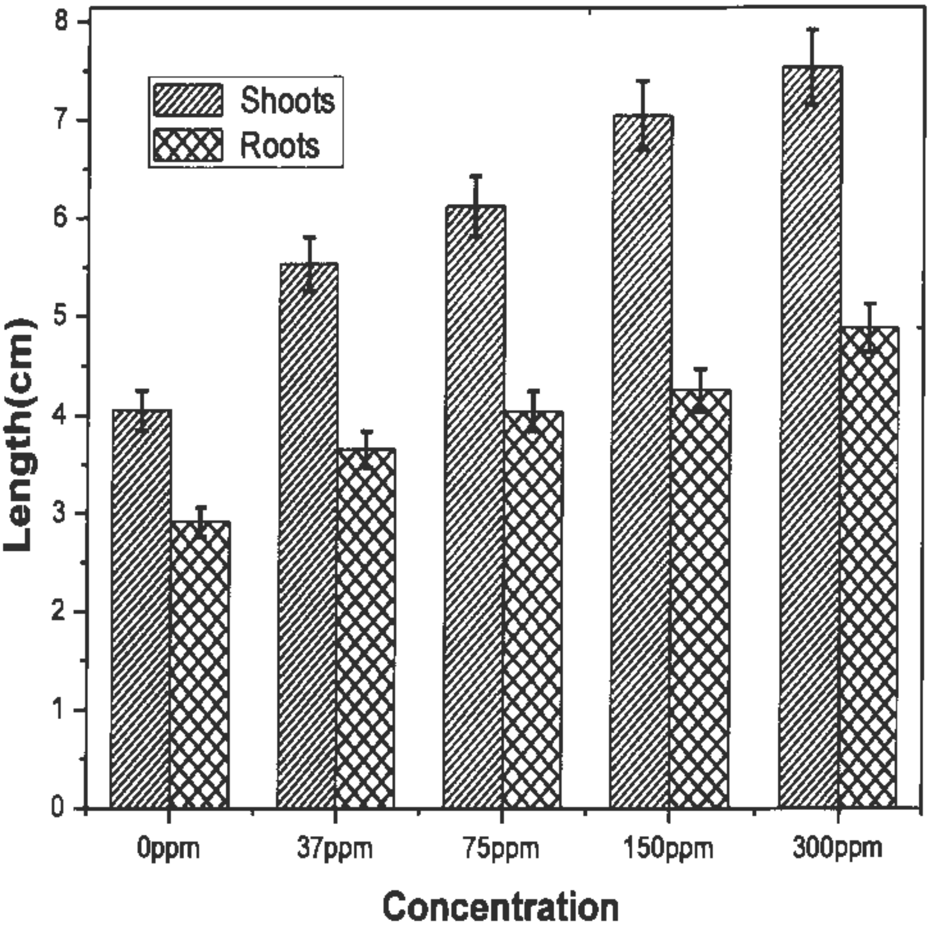


Figure 4.1: Effects of different concentration Ag NPs on wheat (*Triticum aestivum)* shoot and root length, all treated concentration of Ag NPs positive effected on wheat root/shoot growth. All values are mean of triplicates (± S.E., n 3)

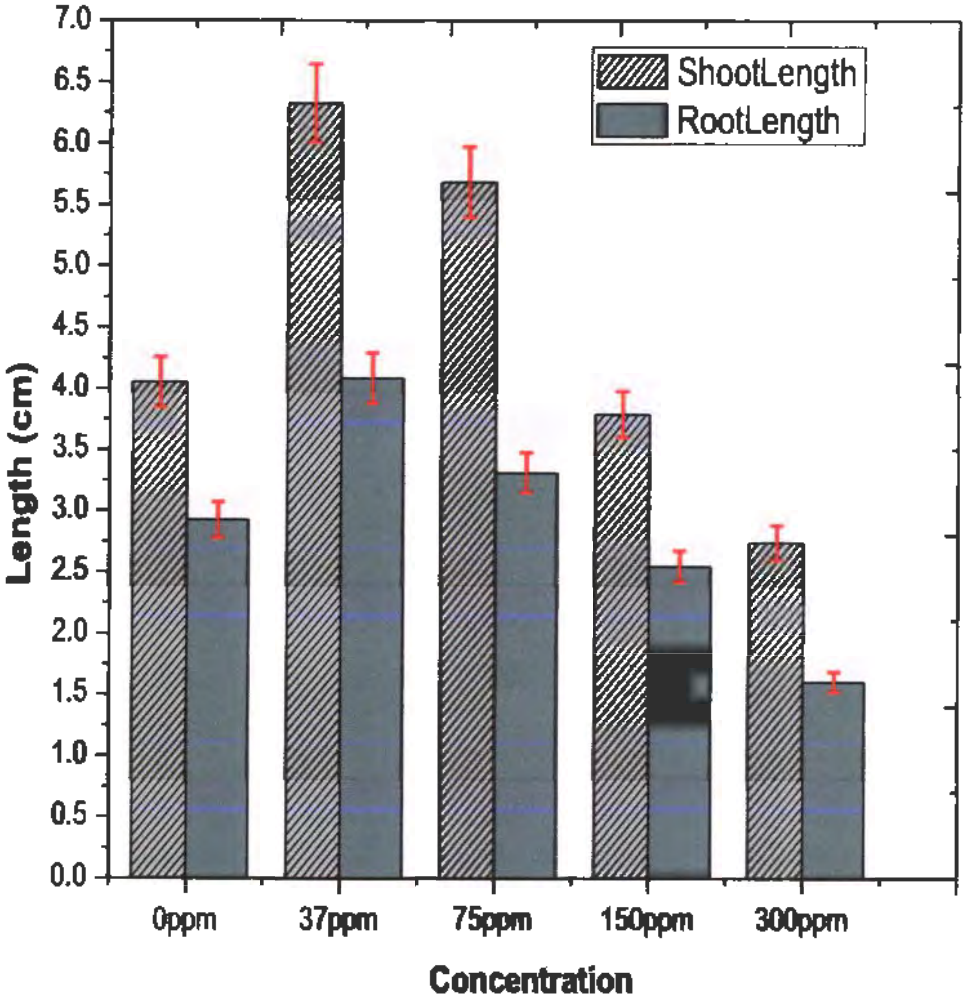


Figure 4.2: Effect ZnO nanoparticles on roots and shoots length. All the value are means of triplicates (± S.E., n=3). Lower concentration (37, 75ppm) have positive effect on shoots and roots growth. Roots/shoots length were increased, at higher concentration (150,300ppm) have negative effect and inhibited the roots/shoots growth. Roots/shoot length were decreased.

4.7. Chlorophyll response to Silver nanoparticles

Chlorophyll is very imported pigments because photosynthesis occurs in chlorophyll and all the livening creature depended on photosynthesis directly or indirectly. In research work we were reported that silver nanoparticles were positive effected on chlorophyll contents. As the concentration of silver nanoparticles increased the chlorophyll contents also enhance. C Pandey et al in 2014 state that silver nanoparticles could significantly promoted the photosynthesis and also change the nitrogen metabolism (Pandey *et al.*, 2014).

From the results of our research work we were reported that at all the four concentrations of Ag NPs increased the chlorophyll contents in *Triticum aestivum* plants (Figure 4.3). At lower concentrations (37ppm and 75ppm) the chlorophyll contents were increased 12% and 16% as compared to untreated plant. While at higher concentration 150 and 300ppm chlorophyll contents were increased by 25% and 36% respectively as compared to control one wheat plant.

4.8. Chlorophyll response to Zinc oxide nanoparticles

It was reported that zinc oxide nanoparticles could significantly interfere with nitrogen metabolism and also demoted the photosynthesis of the plants (Wang *et al.*, 2015).

In the current study we were reported that all the ZnO NPs treated *Triticum aestivum* plants were greatly affected. Figure 4.4 showed that at all concentration of ZnO NPs the chlorophyll contents were decreased and down peak were seen. At 300ppm and 150ppm treated ZnO NPs plants were greatly affected and down peak were shown, while at 75ppm and 37ppm was moderately negative affected and down spectrum were observed. Figure 4.4 also showed that the untreated (0ppm) wheat plant was not affected and the chlorophyll was normal in range and the spectrum also in normal peak.

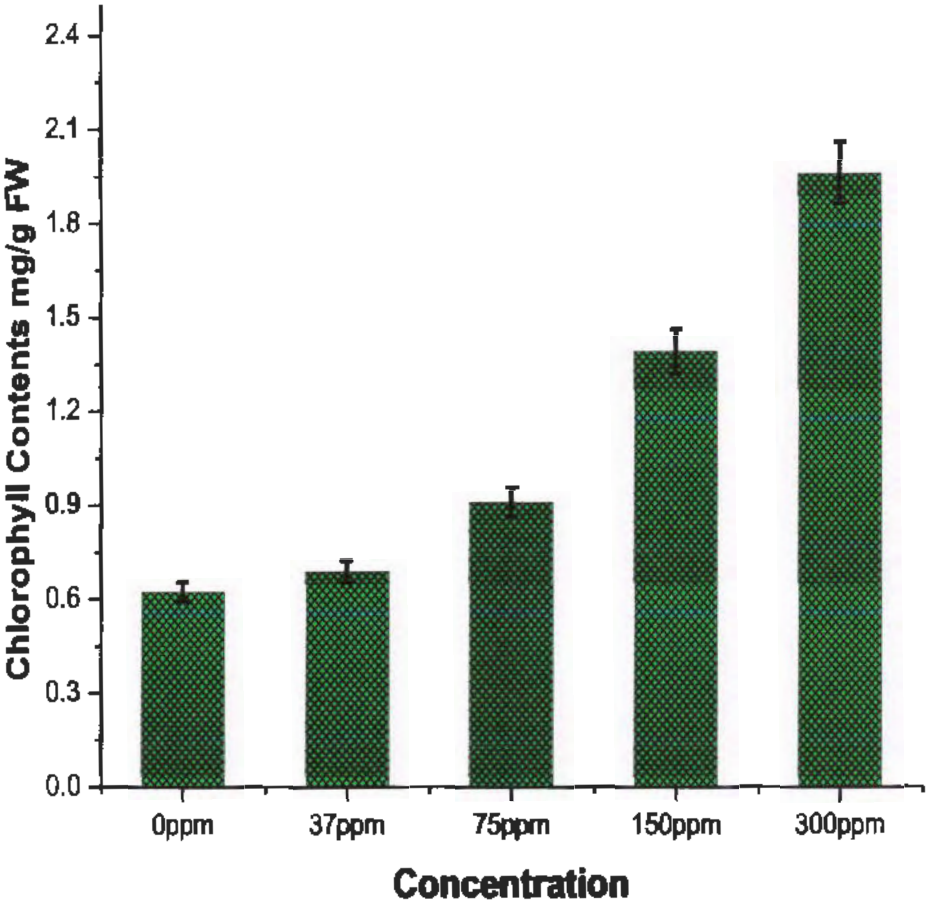


Figure 4.3: Spectrophotometeric analysis of chlorophyll showed that all treated concentration of Ag NPs increase chlorophyll contents. i.e. Ag NPs positive effected on chlorophyll contents of *Triticum aestivum* plants. (Means of triplicates ± S.E.., n=3)

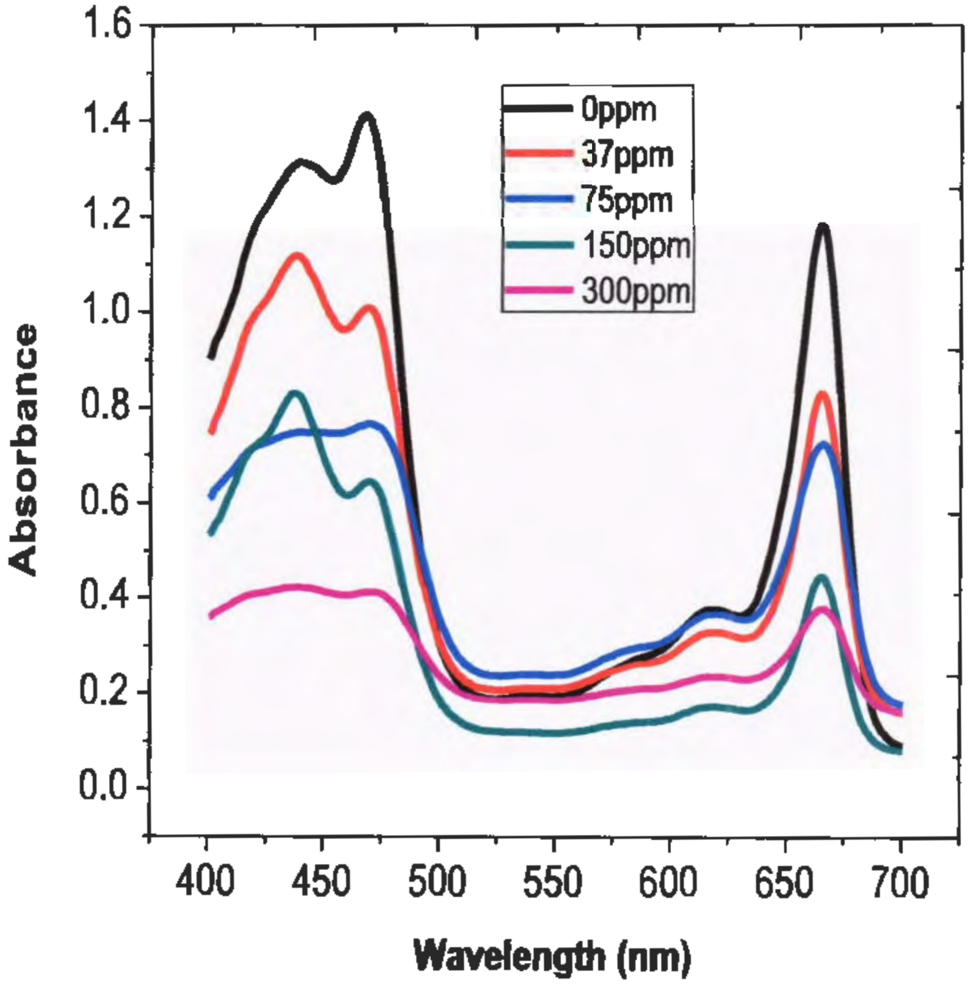


Figure 4.4: Effect of ZnO nanoparticles on chlorophyll contents. All the concentrations of ZnO NPs were having a negative effect on chlorophyll contents. 300ppm and 150ppm were more toxic to chlorophyll contents and down spectrum were seen; similarly 37 and 75ppm were also having negative effects.

4.9. Protein response to Silver nanoparticles

Lu et al in 2001 reported that silver nanoparticles could enhance the germination, plants growth and prevent it from moldy in Glycine max (Lu *et al.*, 2001). In our current research study we reported that silver nanoparticles at all concentration were positive affected on protein contents of wheat plants. At all concentrations silver treated wheat plants were showed that the total protein contents were enhanced as compared to control one. In figure 4.5 shown that at all concentration of silver treated plants proteins contents were increased as compared to control one. At 300ppm and 150ppm the protein contents in shoot were increased by 30% and 25% respectively as compared to untreated wheat plants. Similarly at 75ppm and 37ppm total protein contents in the shoots of silver treated wheat plants were increased by 18% and 15% as compared to untreated *Triticum aestivum* plants.

4.10. Protein responses to zinc oxide nanoparticles

The effects of zinc oxide nanoparticles on protein contents on wheat plants were different. Lower concentration of ZnO NPs promoted the total protein contents in *Triticum aestivum* plants, while higher concentration of ZnO NPs decreased protein contents in *Triticum aestivum* plants. Figure 4.6 illustrate that a significant increased in protein contents were found when wheat plants were exposed to lower concentration of ZnO nanoparticles. About 5.5% at 37ppm and 4% at 75ppm increased were reported in protein contents of *Triticum aestivum* plants. While at higher concentration the results were opposite and the protein contents were decreased. At 300ppm ZnO NPs treated wheat plants showed 5% decreased protein contents as compared to untreated wheat plant, while at 150ppm ZnO NPs treated wheat plants showed 2% decreased protein contents as compared to untreated wheat plant.

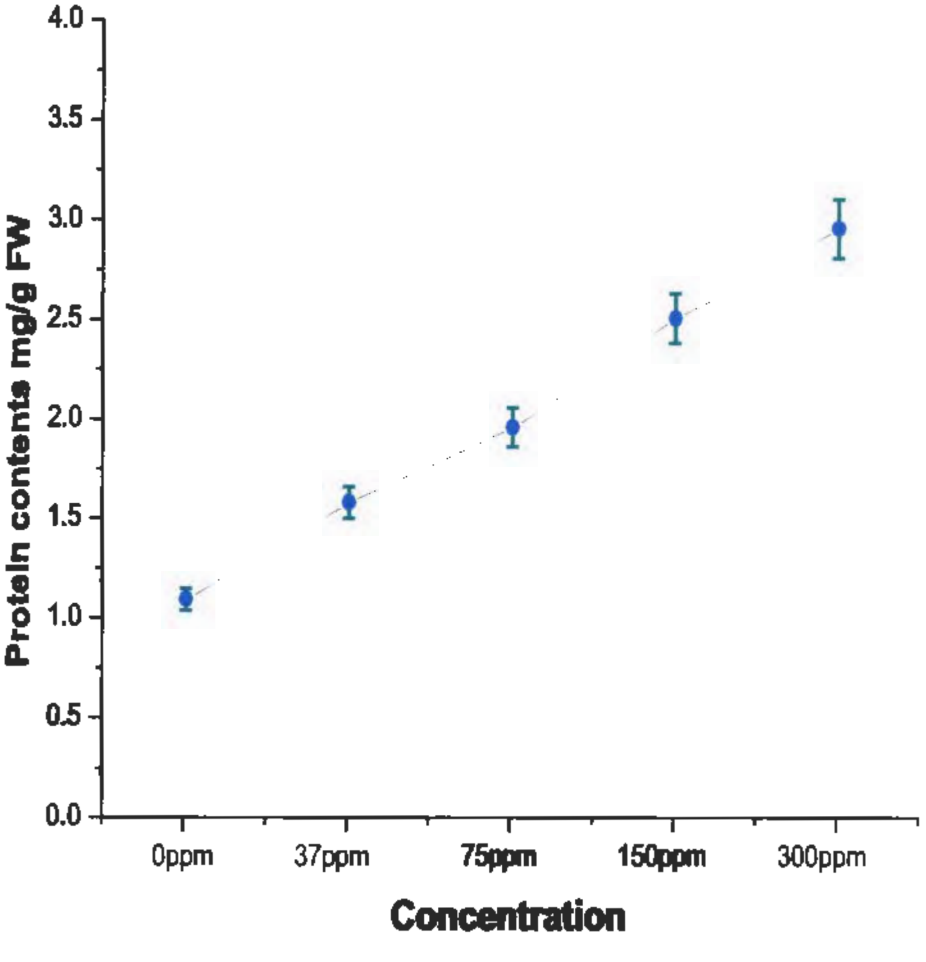


Figure 4.5: Effects of various concentrations of Ag NPs on protein contents in leaves of *Triticum aestivum*. All treated concentration of Ag NPs enhances protein contents. All values are mean of triplicates (± S.E., n=3)

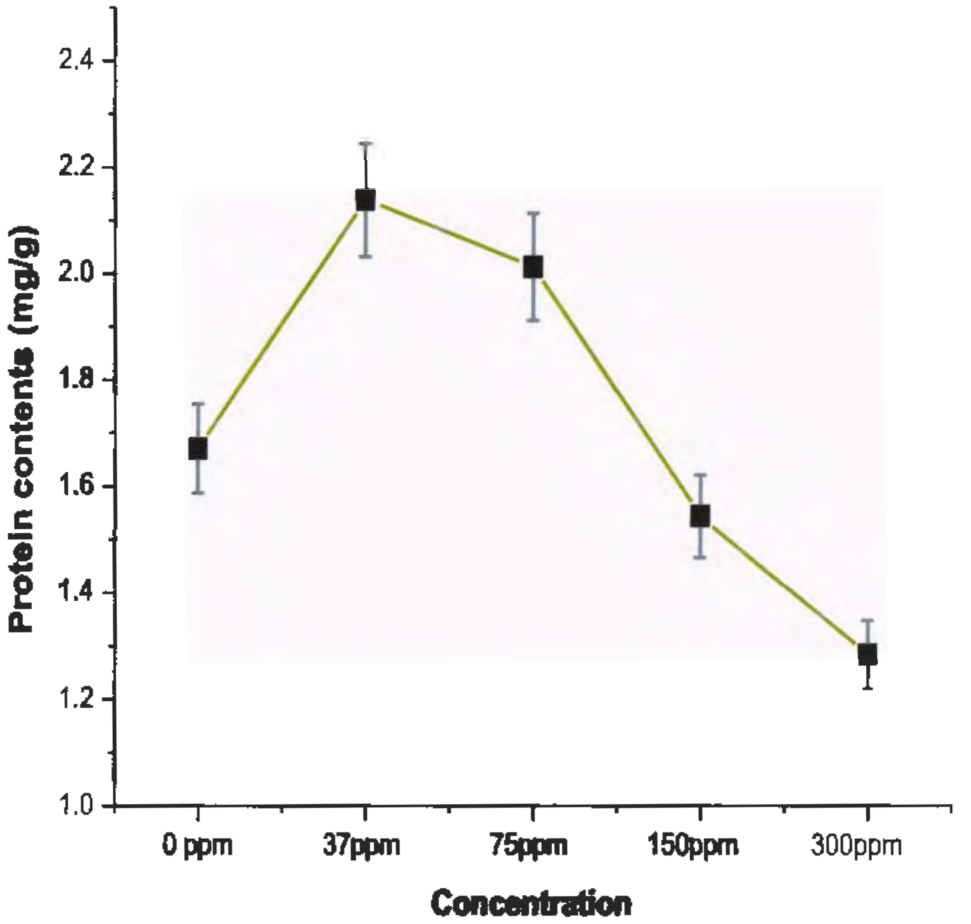


Figure 4.6: Effect of different concentration of ZnO nanoparticles on protein contents in leaves (means of triplicates ± S.E.., n=3). Lower concentration of ZnO NPs increased the protein contents while higher concentration negative effected the protein contents and reduces the protein contents

4.11. Lipids degradation by silver and zinc oxide nanoparticles

Silver is most imported element for plants growth and development. It is also known for its antimicrobial behaviors and shoots regeneration properties and also known for toxic nature to cell and cell physiology (Zhang *et al.*, 2005). Similarly zinc is also imported elements for plants growth and development. The phytotoxicity of ZnO nanoparticles were also reported on *Arabidopsis* plants cell membrane (Lee *et al.*, 2010).

Like many others metabolites lipids also exist in dynamic state in plants i.e. at one time they are being synthesized and at other times broken down to meet specific requirement of the cell. In our research work we were reported that Ag and ZnO nanoparticles were toxic for wheat lipids and lipids degradation were studied through FT-IR analysis. At all treated concentration of silver and zinc oxide nanoparticles, lipids degradation were reported. There are special degradative enzymes found in specific region of plants tissues or found in specialized organelles. These enzymes are responsible for lipids degradation in plants cell. These enzymes are over excreting during environmental stress. Change in ionic strength, PH and others environmental stress can increase the chance of lipids to enzymatic attack. When some environmental stress attacks the plants cell these degradative enzymes form "enzymes substrate complex" with lipids and as a results the lipids shaped are changed and degraded (Quinn & Williams, 1979). It is anticipated that such phenomena, might be happening in the current condition. At all treated concentration of silver and zinc oxide nanoparticles lipids degradation were reported in FT-IR analysis. Higher concentration of Ag and ZnO nanoparticles treated wheat plants more effected then lower concentration and more lipids degradation were observed and large stretching vibration were seen through FT-IR analysis. Lower concentration (37ppm and 75ppm) of Ag and ZnO nanoparticles were also toxic for wheat lipids but as compared to higher concentration (300ppm and 150ppm) their effect were less and less degradation were reported and small stretching vibration were seen through FT-IR analysis. Actually the stretching vibration composed by lipids functional groups, such is OH group (Phenol and Alcohol), COOH (Carboxylic Acid), C-H (Alkane), --C=C-- (Alkenes), C≡ (Alkynes) and N-O (Nitro Compound).These functional groups indicted that silver and zinc oxide nanoparticles adverse effected on wheat lipids and degraded into its relevant functional groups. In figure 4.7A and figure 4.7B had shown all the

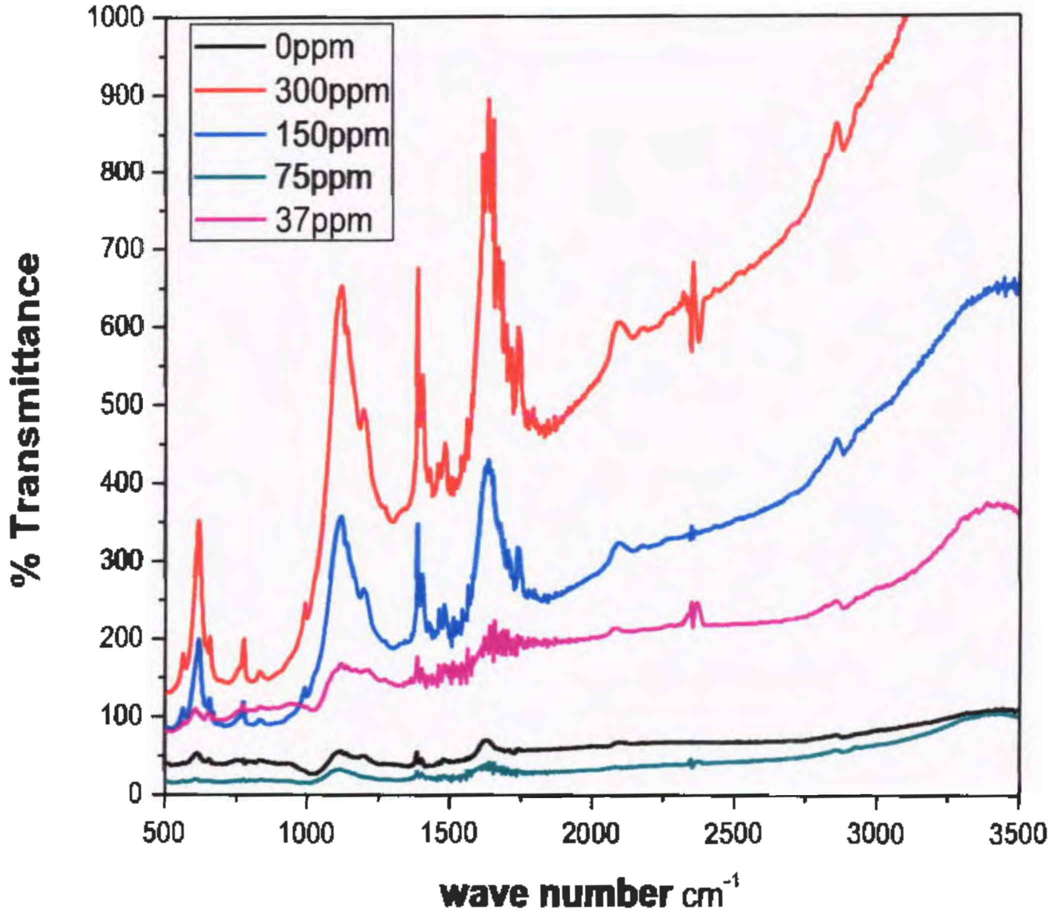


Figure 4.7B: FTIR analysis of wheat plants lipids. All treated concentration of ZnO NPs adverse affected on lipids and lipids degraded into its functional groups. In figure showed that higher concentration degraded more lipids and large stretching band were seen as compared to lower concentrations of ZnO NPs. Non treated plant (0ppm) indicted by black line showed no stretching band so lipid degradation were not happen.

4.12. Proline response to Silver nanoparticles

Proline is specific amino acid plays very vital role in plants, when plants are exposed to various stress conditions. There are effective correlation between proline accumulation and plant stress conditions. Plants over excrete proline during stressful environments which turn propagate stress tolerance by maintaining cell osmotic balance, cell turrgor and stabilizing the membrane. So preventing electrolyte leakage and import the concentration of reactive oxygen species (ROS) within optimum range, therefore avoids oxidative burst in plants (Hayat *et al.*, 2012).

In our research work, we also study proline accumulation in silver nanoparticles treated *Triticum aestivum* plants and one untreated *Triticum aestivum* plants. From the results we reported that very small amount of proline were accumulated in treated *Triticum aestivum* plants as compared to untreated plant. At 37ppm 4%, at 75ppm 4.1%, at 150ppm 4.5% and 300ppm 4.6% proline were reported as compared to control one plant. Figure 4.8.

4.13. Proline response to Zinc oxide nanoparticles

Biochemical analysis of proline in roots and leaves of ZnO NPs treated *Triticum aestivum* plants showed that there was a fluctuation in proline accumulation of various concentrations of ZnO NPs treated plants. At lower concentrations of ZnO NPs small amount of proline accumulations were reported while at higher concentrations more proline accumulations were reported in *Triticum aestivum* roots and leaves. It was also reported that the level of proline accumulation in *Triticum aestivum* leaves less then proline accumulations in *Triticum aestivum* roots. Proline analysis were shown in figure 4.9 at 300ppm nanoparticles treated plants proline level increased by 2.7 folds in leaves while 3.3 folds in roots. At 150ppm proline level increased by 2.3 and 2.8folds in wheat leaves and roots respectively than untreated wheat plants. Similarly at 75ppm proline level increased by 2folds in leaves and 2.7folds roots, while at 37ppm proline levels were increased by 1.5folds and 2.1folds in leaves and roots of *Triticum aestivum* plants as compared to untreated plants.

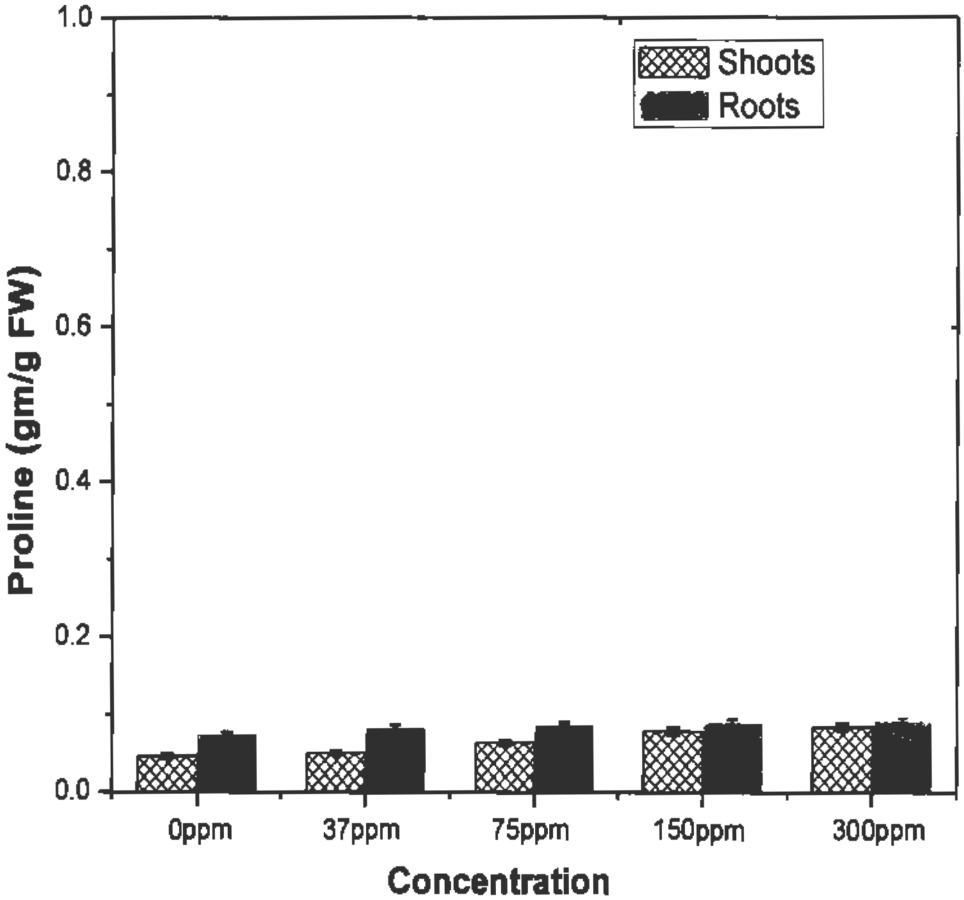


Figure 4.8: Effects of Ag NPs on proline of wheat root and shoot very small amount of proline were reported with treated concentrations of Ag nanoparticles..

(Means of triplicates ± S.E.., n=3).

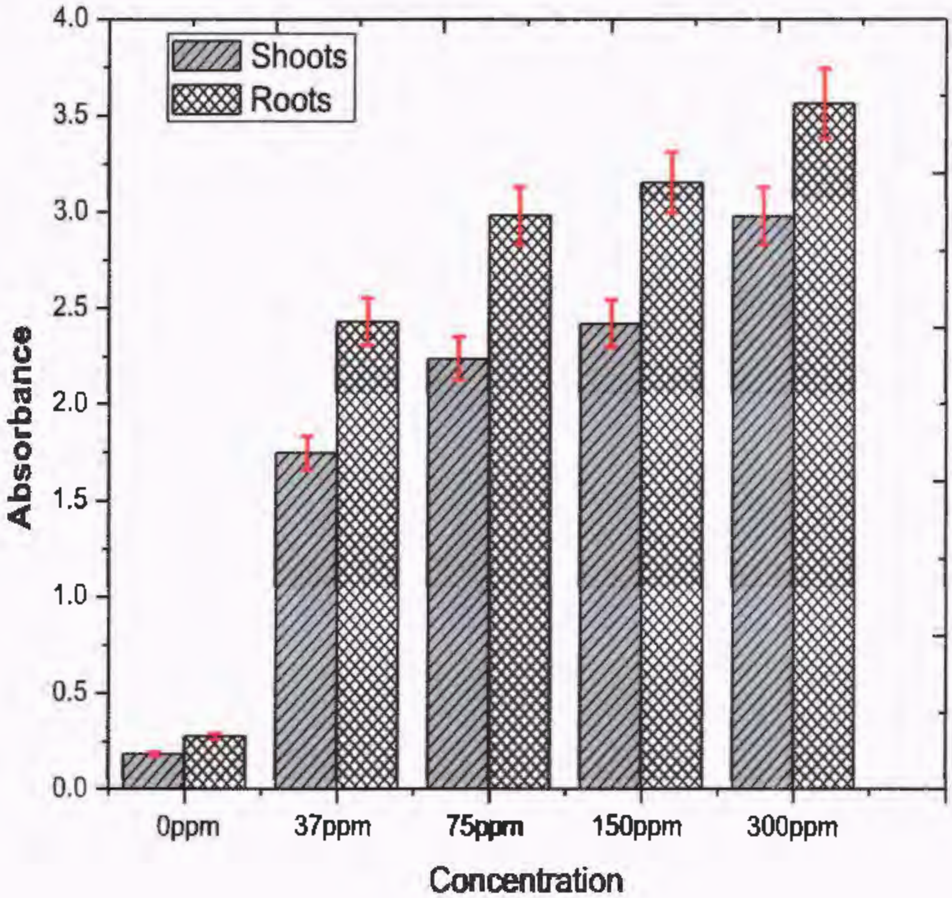


Figure 4.9: Proline accumulation in the leaves and roots of wheat grown under different concentrations of ZnO nanoparticles (Means of triplicates ± S.E.., n=3). At higher concentration of ZnO NPs more proline execration were occur. As the concentration decrease from 300ppm to 37ppm proline execration were also reduce.

Table 4.1: Effected of Silver Nanoparticles on *Triticum aestivum* Plant

AgNPs Concentrations	Root/Shoot Length	Chlorophyll Contents	Protein Contents	Lipids Degradation	Proline Responses
37ppm	Increased	Enhanced	Increased	Degraded	Small amount proline were reported
75ppm	Increased	Enhanced	Increased	Degraded	Small amount proline were reported
150ppm	Increased	Enhanced	Increased	Degraded	Small amount proline were reported
300ppm	Increased	Enhanced	Increased	Degraded	Small amount proline were reported

Table 4.2: Effected of Zinc Oxide Nanoparticles on *Triticum aestivum* Plant

ZnO NPs Concentrations	Root/Shoot Length	Chlorophyll Contents	Protein Contents	Lipids Degradation	Proline Responses
37ppm	Increased	Decreased	Enhanced	Degraded	Over Excreted
75ppm	Increased	Decreased	Enhanced	Degraded	Over Excreted
150ppm	Decreased	Decreased	Declined	Degraded	Over Excreted
300ppm	Decreased	Decreased	Declined	Degraded	Over Excreted

Chapter. 0 5

CONCLUSION AND RECOMMENDATIONS

5.1. CONCLUSION

In our research work the impact of different concentration of silver and zinc oxide nanoparticles were studied on root and shoot length and different biochemical parameters of *Triticum aestivum* plant in the lab using optimum light condition. The concentration levels 37, 75, 150 and 300ppm were applied to wheat plant. In case of Ag NPs all the treated concentrations were positive effected on wheat plants root/shoot length, protein and chlorophyll contents. As the concentrations of silver nanoparticles were increased from 37ppm to 300ppm, the root/shoot length, protein and chlorophyll contents also increased. In case of lipids Ag NPs were negative affected and Ag NPs were toxic for wheat plant lipids and lipids degradation were reported at all concentration of Ag NPs, higher concentration of Ag NPs is more toxic and more lipids degradation were reported as compare to lower concentration.

The results of zinc oxide nanoparticles were different on wheat plants. Lower concentrations were positive effected on wheat plants root, shoots and protein contents. At 37 and 75ppm the wheat plants shoots, roots and protein contents were increased. But higher concentrations of ZnO NPs negatively affected and decreased were seen in wheat roots, shoots and protein contents. While in case of chlorophyll and lipids ZnO NPs were negative effected. From 37 to 300ppm concentrations of ZnO NPs, chlorophyll contents were decreased. More lipids degradations were reported on 300ppm and same way 150ppm, 75ppm and 37ppm also toxic for lipids and lipids degradation were reported. The result of proline of root and shoot were also showed that at higher concentration more proline was excreted as compare to lower concentration of ZnO NPs.

5.2. RECOMMENDATION

Nanotechnology boosted in many fields of sciences, so nanotechnology is getting more and more attention and expanded in different applications. It can be critical or crucial for the agriculture and environment in future. The present study point out some the potential effects of nanoparticles but still there is a long way to go. The experiment time was short, so there is need to investigate the impact of nanoparticles over longer periods. It would be perfect to observe what happen with grain formation, effects on plants metabolisms and quality of products. If there are no changes in plants physiology then it would be interesting to changes the dosage of nanoparticles i e increase or decrease. The ranges used in current study were different, so these should be narrowed to find out point out exact for beneficial and toxic effects due to nanoparticles applications. Further studies are required to use these nanoparticles in combination with different levels of fertilizers and micronutrients.

It is well reported in the literature that the laboratories experiment can provide some information about real scenario but not the real picture; therefore also need to conduct experiments in actual soil conditions, soil structure and texture may have various effects of the availability of different nutrients and the role of nanoparticles. Under some stress conditions such as drought and salinity the nature of nanoparticles could be complex. In additions to all above mentioned scenario, mechanisms of uptake by plants from soil, localizations within plants need to be carefully studies.

Apart from the potential benefits of this kind of work there is also some restriction that could not ignore. At this stage we could not [illegible] with surety that this kind of technology is fully safe for human health and environment or harmful. Risks are associated with persistent exposure of human of these nanoparticles, [illegible]raction with flora and fauna and their possible bioaccumulation effects have not b[illegible]lly considered yet. Therefore, these concerns should be considered seriously before appl[illegible] this study from laboratories to the field. The other limitations include the safe rang[illegible]anoparticles concentration, scalability of research and about health and safety issues. In [illegible]amework extensive research is necessarily required to resolve these concerns and provide [illegible]table work.

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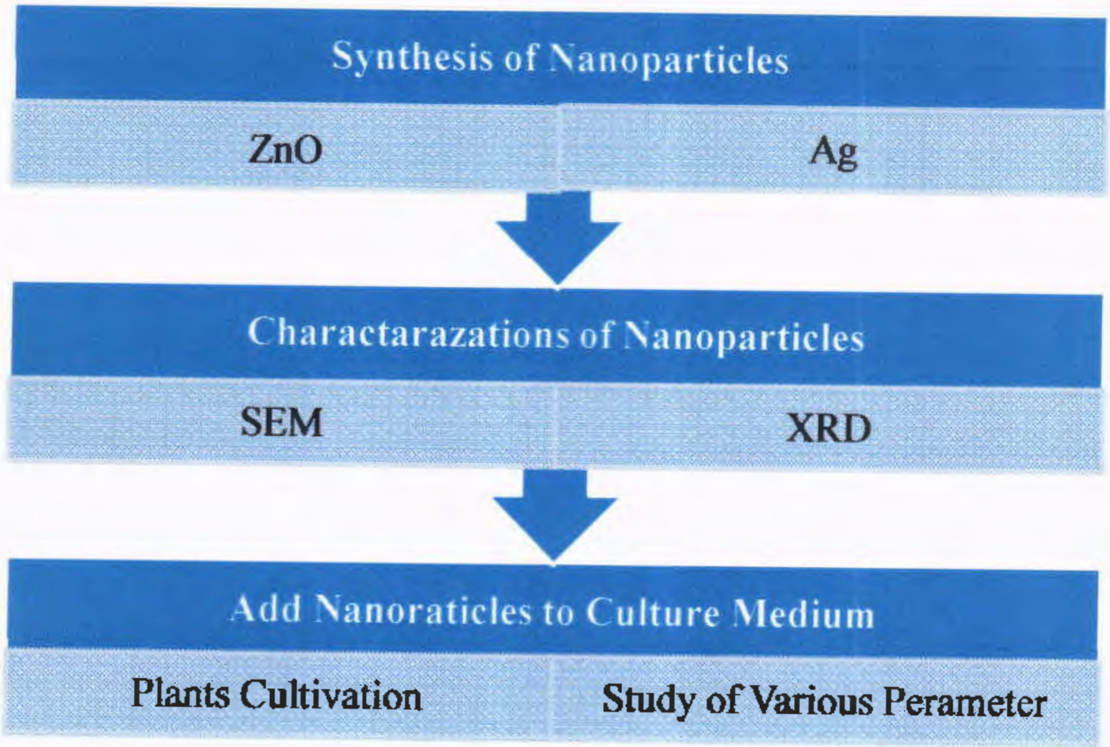
ANNEXURE I

EXPERIMENTAL SETUP



Entry of nanoparticles to plants root

ANNEXURE II

Response of Ag nanoparticles to the Shoot length of Wheat

Concentrations of Ag NPs	Shoot Length (cm)	Standard Deviation
0ppm	4.05	0.553553
37ppm	5.535	1.045429
75ppm	6.12	1.13258
150ppm	7.045	0.946726
300ppm	7.535	0.67767

Response of Ag nanoparticles to the Root length of Wheat

Concentrations of Ag NPs	Root Length (cm)	Standard Deviation
0ppm	2.92	0.842677
37ppm	3.655	0.733036
75ppm	4.04	0.563261
150ppm	4.255	1.014357
300ppm	4.88	1.02166

The values in the table are given as Mean ± SD (Standard Deviation).

ANNEXURE III

Response of ZnO nanoparticles to the Shoot length of Wheat

Concentrations of ZnO NPs	Shoot Length (cm)	Standard Deviation
0ppm	4.05	0.553553
37ppm	6.325	0.970553
75ppm	5.68	1.183927
150ppm	3.785	1.334275
300ppm	2.735	0.930916

Response of ZnO nanoparticles to the Root length of Wheat

Concentrations of ZnO NPs	Root Length (cm)	Standard Deviation
0ppm	2.92	0.842677
37ppm	4.08	0.828188
75ppm	3.305	0.677825
150ppm	2.54	0.778257
300ppm	1.6	0.455377

The values in the table are given as Mean ± SD (Standard Deviation).