Applicability of Silver Nanoparticles for Increasing the Bioavailability of Phosphorus Fertilizer

Dissertation –II submitted by

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To

Department of Biosciences

In the partial fulfillment of the Requirement for the Award of the Degree of

M.Sc. Botany (Hons.)

Under the guidance of

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DECLARATION

I hereby state and declare that the project entitled, "**Applicability of silver nanoparticles for increasing the bioavailability of phosphorus fertilizer**" submitted for the M.Sc. degree this is entirely my original work and all ideas and references have been duly acknowledged. This report has not submitted to any other university, fellowship or institute the basis for the award of any degree or diploma**.**

Date -04-2015 Signature and Name of Student

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CERTIFICATE

This is to certify that Sakshi Naryal has completed M.Sc. dissertation proposed titled, "Applicability of Silver Nanoparticles for Increasing the Bioavailability of Phosphorus fertilizer" under my guidance and supervision .To the best of my knowledge, the present work is the result of her original investigation and study. No part of the dissertation proposal has ever been submitted for any other degree or diploma.

The dissertation proposal is fit for the submission and the partial fulfillment of the conditions or the award of M.Sc. Botany

Date: -04-2015

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Date-

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INDEX

TITLE	PAGE No.
DECLERATION	2
CERTIFICATE	3
ACKNOWLEDGEMENT	4
LIST OF TABLES	6
ABSTRACT	8
INTRODUCTION	9
TERMINOLOGY	13
REVIEW OF LITERATURE	15
SCOPE OF THE STUDY	21
OBJECTIVES OF THE STUDY	23
MATERIAL AND RESEARCH METHODOLOGY	25
RESULT AND DISCUSSION	30
CONCLUSION AND FUTURE SCOPE	37
REFERENCES	39

LIST OF TABLES

	Details of Tables	Page no.
1.1	Treatment of different amount of fertilizers with different concentration of nanoparticles	27
1.2	List of chemicals with quantity required	28
1.3	List of equipments	29
1.4	Concentration of 10 g of phosphorus fertilizer at 50ml, 100ml, 200ml.	32
1.5	Concentration of 25g of phosphorus fertilizer at 50ml, 100ml, 200ml.	33
1.6	Concentration of 50g of phosphorus fertilizer at 50ml, 100ml, 200ml.	33
1.7	Concentration of different samples of chilli	34

ABSTRACT

Nanotechnology has many practical applications in various fields. Nanoparticles or nanocluster or nanocrystals are microscopic particle with at least one dimension less than 100nm. Due to their microscopic nature these particle finds many practical applications in main area of scientific research. Different types of nanoparticles like copper, zinc, titanium, magnesium, gold, alginate and silver have come up but silver nanoparticles are proved to be most effective. From many years silver have many practical applications in biosciences, medicines but modification of silver by nanotechnology leads to increase in efficiency. Besides having many positive effects it has negative impact also. Plants are the main component of ecosystem. NPK are three most important nutrient required by plants for greater efficiency. Phosphorus is second in importance to plants. Transfer of energy is one of the main roles of Phosphorus in living things. Adequate Phosphorus availability for plant stimulates early plant growth and delays maturity. The simplest phosphate is orthophosphate. The negative charge on the orthophosphates prevents it from being inhibited by the soils cation exchange capacity and it reacts strongly in the soil and becomes unavailable to plants. In the current study the investigation is done on the applicability of silver nanoparticles on increasing the availability of Phosphorus fertilizer. The result after experiments shows that silver nanoparticles have positive effect on the uptake of Phosphorus fertilizer.

CHAPTER-1

INTRODUCTION

Introduction

With the invention of new technologies in the field of nanosciences, living beings, plants have various advantages. Nanopowders, nanocrystals, find very diversifying role in electronics, medicines, chemistry and physics. Green synthesis is regarded as best cost effective method for nanoparticles synthesis. It leads to the use of plants product thereby results in an ecofriendly method for nanoparticles synthesis. Various scientists have worked on the nanoparticles till now. Nanoparticles, nanocrystals are the particles having structure range between 1 to 100 nm **(Wigginton et al., 2007)**.

Nowdays nanoparticles are of great importance. This is due to the reason that they possess almost different properties when compared to the same material present in the bulk state.This happens because of nanoparticles surface to volume ratio. Being heterogeneous nanoparticles consist of two parts, the inner core material and outer surface. The core of nanoparticles is different from outer surface. Core is classified as the main body of nanoparticles. Nanoparticles are named on the basis of useful applicability's of core material **(Christian et al., 2008)**.

Surface of nanoparticles accounts for the overall function of nanoparticles **(Perez et al., 2009)**. Third part of the nanoparticles is shell, supposed to be more reactive. Nanoparticles due to their excessive use are supposed to reach water bodies in few years. Due to the interaction of the nanoparticles with various substances present in water bodies and aquatic animals, nanoparticles may think to exert the toxic substances **(Chakraborty, 2009)**

Nanoparticles are also used as protecting device in living systems. In plants nanoparticles are used to control the infections. Nanoparticles to reduce plants disease, formed from secondary metabolites **(Hameed and Samarrai, 2012)**

The use of nanoparticles as transporting agent was initially explored in medical areas. This transporting nature of nanoparticles is useful in plants. Nanoparticles can reached to specific areas in plant parts without causing any hazardous effect. Nanoparticles combined with chemicals in order to target a particular plant part. This method requires careful and proper lancing and delivery of nanoparticles to the plant parts **(Melendi et al., 2008)**

Silver nanoparticles are prepared by various methods. Plant extract used for the synthesis of silver nanoparticles. The extract of the plants functions as reducing agent. Silver nitrate was added to plant extract result in the formation of nanosized silver **(Sivaraman et al., 2009).**

Silver nanoparticles have gained the attention of many researchers due to their distinctive properties. Silver nanoparticles show activities against bacteria, which provide a cheaper way for the development of antibacterial agents **(Calinescu et al., 2011).**

A silver nanoparticle creates reactive oxygen species (ROS) by damaging the cell membrane. The change in cell membrane causes cell membrane more permeable to silver ions and Silver nanoparticles **(Bae et al., 2011).**

Phosphorus is one of the macromolecule needed by plant for better growth and development. Phosphorus is an inorganic compound. Phosphorus is needed by all plants for the manufacture of nucleic acids, lipids of membranes and ATP. Deficiency of Phosphorus arises in soil, if it the land is repeatedly cultivated or used for crops. Improper application of phosphorus can causes hazardous effect on soil, plant etc. If any element is present in lower concentration in soil as compared to the needed concentration, plants results in slower growth rate (Environmental dormancy). To avoid such discrepancy proper amount of element is added in soil. Phosphorus deficient plant bears reddening of leaves **(Scott)**

Not only the lower concentration of Phosphorus, but excess Phosphorus in plants also dangerous. As a rule both macronutrient as well as micronutrient is important to plants. Larger concentration of Phosphorus than normal also creates problem. This will also affect the mycorrhizal infection. Decrease in normal count of mycorrhiza results in extra efforts by the plants. **(Scott)**

The negative charge on the orthophosphates prevents it from being inhibited by the soils cation exchange capacity (CEC) it does react strongly in the soil and mainly with the large amount of
Iron and aluminum naturally in the soil, and becomes unavailable to the plants by forming insoluble products and thus unavailable to the plants.

Thus keeping in view the utility of nanoparticles in so many fields, it has been hypothesized that applicability of nanoparticles must be explored, and will provide great insights into increasing the solubility of P- fertilizers in soil.

Chilli (Capsicum *annum*) is a spice. It is considered as spice as well as vegetable crop. Chilli is grown almost in every part of India. Chilli is considered as an important constituent in Indian food. Around 7.67 lakh hectares area is occupied for the cultivation of chilli in India. India accounts for about 12.34 lakh tones. Andhra Pradesh solely account for 35% area under cultivation of chilly in India. In the year 2007-08 India becomes the larger supplier of chilli in International Market. Countries like China, Pakistan suffered from loss of chilli. This will help India to become larger supplier. **(Reddy et al., 2011)**

CHAPTER-2

TERMINOLOGY

Terminology

NP	Nanoparticles
AgNPs	Silver nanoparticles
FE-SEM	Field emission scanning electron microscopy
DLS	Dynamic light scattering
SEM	Scanning electron microscopy
AAS	Atomic absorption spectroscopy
FTIR	Fourier transform infrared spectroscopy
XRD	X-ray diffraction
$AgNO_3$	Silver nitrate
Al-P	Alumina- buffered phosphate
RCBD	Randomized complete block design
ATP	Adenosine tri phosphate

CHAPTER-3

REVIEW OF LITERATURE

Review of literature

NPs or nanomaterials are first used in medical area as a transporting agent to particular area. In case of plants same mechanism is found to be very effective. Generally the mechanism is used in plants to reduce infections. NPs attached to the substances reduce the effect of damage to various plant parts. For the purpose NPs need proper attention. NPs must be penetrated with care to be used as transplanting device in case of plants. **(Melendi et al., 2008)**

Maass, 2008 studied that increased use of NPs have any deleterious effect on environment or not. Silver have efficient antigerm activities and AgNPs are well known due to their high surface area. Many companies use AgNPs for the manufacture of various products. It is supposed that these products when disposed off have any effect on the environment. It is concluded in the study that nanoshaped silver when come in contact with soil and water do not remain as such. These may accumulate to form bigger silver particles that are environment friendly.

NPs are leading future of many growing industries. It play tremendous role in this area. Products that are used in routine life are largely influenced by NPs. Due to the growing demand of NPs they are largely synthesized. But the NPs are thought to create problem in water bodies. Because due to their greater demand they are expected to be reach to the aquatic world. NPs release some toxic compounds that harm the aquatic animals. In spite of releasing toxic substances, NPs can also react with other water substituent's and cause harm. **(Chakraborty, 2009)**

NPs are used as a transporter. The use of NPs as a transporting agent is to minimize the various pathological symptoms of plants. NPs such as AgNPs, Gold NPs are easily manufactured and replaced the pesticides. To reduce the plant disease secondary metabolites are utilized. Secondary metabolites are free of any side effect, having plentiful bioactive chemicals, free of causing pollution, in simple terms environment loving. **(Hameed and Samarrai, 2012)**

This study is advantageous in order to summaries the uptake of NPs at seed germination. The toxic effect of AgNPs is major obstacle during germination period of seed. For the study seeds of rice, mustard and green gram were utilized as test seeds. Chemical reduction method is used for AgNPs synthesis. On increasing AgNPs concentration there is increase in accumulation of AgNPs in test seeds. **(Mazumdar, 2014)**

AgNPs due to their idiosyncratic chemical and physical behavior have many advantages. Moreover the activity of AgNPs towards bacteria provides a tremendous cheaper alternative in the development of many antibacterial agents. **(Calinescu et al., 2014)**

Angelina et al., 2013 formulated an easy method for the synthesis of AgNPs. In this mechanism seed extract of fenugreek were used as reducing agent. When seed extract of fenugreek was added to silver nitrate its color becomes dark brown. The characterization of AgNPs was done by using spectroscopic methods and XRD. The overall mechanism was done at room temperature. In this mechanism we do not use any deleterious chemical therefore considered to be safe.

Unlike green synthesis of AgNPs, chemical synthesis also proved to be very effective. AgNPs are prepared by chemicals. Sodium borohydride and trisodium citrate as reducing agent, for the reduction of salt of silver salt of silver, silver nanocolloidal solution is prepared. Ascorbic acid which is a mild reducing agent is used for the reduction of silver salt, leads to the formation for triangle shaped nameplates of silver. The conformation of nanostructures is done by spectroscopic method and SEM. The total percentage of Ascorbic acid present in various samples of Vitamin-C is determined by using reducing nature of Ascorbic acid. **(Rashid et al., 2013)**

Awwad et al., 2013 described fast environmental mechanism for manufacturing AgNPs. In this method the synthesis of AgNPs is done by green modes. In this mechanism, the AgNPs is manufactured by using solution of silver nitrate. The extract of leaves of *Ceratonia siliqua* is used as a reducing agent. Stable AgNPs are prepared at different concentrations of silver nitrate. The synthesized AgNPs are mostly spherical in shape. The thickness of AgNPs lies in between 5 to 40 nm. *Ceratonia siliqua* leaf extract used as a reducing agent finds easier and time consuming method for the reduction of ions og silver to the nanomaterials of silver. The reaction hardly requires 2 minutes, with normal conditions. Chacterization of AgNPs further done by spectroscopic method, XRD, FTIR, ABS.

Malabadi et al., 2012 studied the green synthesis of AgNPs. It is done by using whole leaf extract of plant named *Clitoria ternatea*. AgNPs activity against bacteria was examined ag *Escherichia coli, Bacillus subtilis, Staphylociccus aureus and Klebsiella pneumonia.* The strains of *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella* may cause poisoning of food. The study purely indicate that the plant extract mediated AgNPs synthesis have many practical applications against these fatal pathogens.

Zhou and Wang, 2012 discovered a basic and cost effective mechanism for AgNPs synthesis. The method requires only one step. In this method sodium citrate works both as capping as well as reducing agent. The process is completed by injecting sodium citrate to silver nitrate solution. High temperature is needed for the process. The method is environment friendly. Sphere shaped AgNPs are formed. Manufacturing is characterized by field emission scanning electron microscopy, dynamic light scattering. Activity of AgNPs on retardation of lung cancer cells of humans was also examined in artificial conditions.

Srivathsan et al., 2012 synthesized the AgNPs and examined their effect on bacterial world in soil. NPs are synthesized from silver nitrate. Wet chemical method is used for synthesis involving metal reductant named Trisodium citrate. Bacteria were isolated from the soil mainly the area which surrounds the root. AgNPs of varying concentration was supplemented on agar plates. The NPs effectively inhibit the bacterial growth. For the reason the NPs are significant as antibacterial systems. This approach og nanotechnology against the against the toxicity of microorganisms present in the soil is beneficial. The reduction in microorganisms is caused by NPs can be recovered by regeneration process. It is always recommended the minor use of AgNPs in agricultural field.

Sivaraman et al., 2009 described convenient, fast, green and most interesting thing economically important method for AgNPs synthesis. The whole mechanism needed room temperature. Plant extract are used as reducing agent. Extract of plant such as tannic acid which is a polyphenolic compound is utilized as a reducing agent in whole process. Silver nitrate is added to tannic acid to which plant extract act as a reducing agent, leads to the formation of nanosized silver particles. The sizes of AgNPs synthesized are 3.3 to 22.1 nm. The size may vary according to the change in concentration of tannic acid and silver nitrate.

Zhang et al., 2002 conducted experiment to evaluate the effect of Al-P on woody plants regarding their growth and tolerance to drought. The effect of Al-P was also examined in establishment of seedling of various trees species. The different species of trees were cultured in containers. Al-P will result in reduction of P-leaching in all species. The desorption value of Al-P is 74 uM. This gives sufficient Phosphorus for proper development and growth of woody plants. In case of **rhododendron**, promotion of flowering is done in first year by Al-P. In case of **forsythia** the Al-P leads to increase in rate of growth even when the plant is suffering from water deficient conditions.

Medicinal plants are valuable to mankind. *Hpericum perforatum* (Saint John wort) is an important medicinal plant used since time immemorial. Medicinal value of plant is due to the production of large amount of secondary metabolites. Important one is **naphthodianthrones, phloroglucionols, flavonoids** etc. In the present study two years field trial is done for study of three levels of Phosphorus and Nitrogen fertilizer. HPLC-DAP are used to determine the flavonoids content. It is concluded that the application of fertilizer leafs to the improvement of drug quality and flavonoid content. **(Azizi and Dias, 2002)**

In recent past conserved cultivation gained the attention due to their perpetual demand whole year. Use of green house is proved to be advantageous. Investigation is done to study the behavior of capsicum varieties under the green house. All the varieties are well adapted under the conditions of green house. The three varieties were grown in polyphone. Out of three varieties the Arka Mohini variety leads to increase in weight of the fruit. As compared to the fruit weight of Arka Mohini the fruit weight also increased. In case of Arka Bharat variety the diameter of fruit was larger on comparison to the Arka Mohini variety. The yield of fruits also increased. The reason behind the increased yield is was increased fruit weight, length and diameter. **(Aruna and Sudagar, 2009)**

Kottegoda et al., 2011 described a mechanism for the release of nitrogen in soil. Plant named *Gliricidia sepium* is used. Hydroxyapatite NPs modified by urea undergoes encapsulation into the soft wood of *Gliricidia sepium.* The release of nitrogen under the composition of nitrogen fertilizer was taken under examination. Soil is taken from three different elevated parts experiences comparison to the fertilizers that are commercially used. The release of nitrogen fertilizer was compared in between the nanofertilizer and fertilizer that are commercially used. The nitrogen fertilizer shows burst in beginning and the movement of fertilizer is very slow. Movement of fertilizer becomes slow on 60 day also. On comparison of this fertilizer to the fertilizer that is used commercially, the movement of fertilizer is less even on 30 day.

Phosphorus due to its larger requirement by the plants regarded as macronutrient. Phosphorus is largely needed by plants for the better growth as well as development. Phosphorus is an essential inorganic compound related with the synthesis of nucleic acid, ATP, lipids. Below normal and above normal concentration of Phosphorus in agricultural land is deleterious. **(Scott)**

Kareen, 2011 conducted experiment on sweet potato to characterize the effect of Phosphorus fertilizer on vegetable growth, tuberous yield and Phosphorus uptake. For present study shaba a variety of sweet potato is

studied. Vegetative growth, yield and uptake of Phosphorus is studied of the plant by four different fertilizer using RCBD. Single super Phosphate was considered to be best.

In vitro the effect of silver nitrate and cobalt chloride on flowering in case of in *Capsicum frutescens* investigated. Effect of cobalt chloride and silver nitrate was also seen on the multiplication of shoot. Best result is seen on external application of both silver nitrate and cobalt chloride at 30 uM of concentration. The positive response is seen by increase in length of shoot and number of shoots. It was investigated after 45 days by culturing on MS media. After 25 days and 45 days both silver chloride at concentration 40uM and cobalt chloride at 30 uM influence in vitro flowering respectively. The results of the experiment found to be very effective in order to transform the capsicum. **(Sharma et al., 2007)**

Production of secondary metabolites their utility and their applicability are the current area of interest of many scientists. Capsaicin is the one of many well known compounds that is found naturally. Recently many researchers are actively focused on the impact of capsaicin on human processes and as a biopesticide. Use of secondary metabolites in various fields such as medicines rapidly grows due to study of various chemicals of *Capsicum annuum*. The Capsicum is used for the extraction of the capsaicin and utilization of this capsaicin as pesticides against Myzus persicae. Spectrophotometrical method is used for calculation of capsaicin content. The ability of capsaicin as a biologically derived pesticide was determined according to Abbott. The oleoresin from Capsicum annum is most effective biopesticide. The oleoresin on its dilution at 1:20 ratio proves to be most powerful pesticide. The Capsicum due to their larger concentration and applicability can be utilized for the effective capsaicin extraction. **(Gudeva et al., 2013)**

Hot sauce, chilli sauce, pepper sauce and various other wide varieties of products are prepared from pepper in all over the world. Vitamin C as well as carotene content of red chilli are very high. Various studies proved the protective effect of capsaicin against stomach ulcers infected with H. pylori. Capsaicin shows anti ulcers properties by affecting chemicals that are released by the stomach in response to infection. Rats were used to further conduct the experiment. In this study the effect of extract of red pepper and spicy foods on the treatment of gastric ulcers that is induced by aspirin in rats. Result found the practical application of red chilli in treating acute gastric ulcer disease. **(Omar et al., 2013)**

CHAPTER - 4

RATIONALE AND SCOPE OF THE STUDY

Scopes of study

Phosphorus is second in importance to the plants as compared to the nitrogen fertilizer. The phosphorus must be changed into the available forms because of its low availability. To increase the availability of the Phosphorus in case of rock phosphate it is treated with acid. Therefore to ensure phosphorus content and availability in the fertilizers laws have been passed. This law requires the manufacturer to state the amount of available phosphorus the fertilizer contains. Phosphorus fertilizer totally soluble in water when it is prepared but does not remain as such for long period after it is applied to the soil. This process of the available phosphorus being made unavailable to the plants is called phosphorus fixation. Therefore main scope of study is to make available the phosphorus to the plant which was not completely available to the plant because of phosphorus fixation that is the process in which the available phosphorus being made unavailable to plant. The silver nanoparticles are of great importance in research field, as it is used for the purpose of increasing the bioavailability of the phosphorus fertilizer.

CHAPTER - 5

OBJECTIVES OF THE STUDY

Objectives of the study

1. Synthesis of silver nanoparticles by plant mediated silver nanoparticle.
2. Use of the silver nanoparticle to increase the bioavailability of phosphorus fertilizer, by changing the molecular structure of the phosphorus fertilizers.
3. Evaluation of uptake of Phosphorus fertilizers by plant system upon treatment with Ag nanoparticles.

CHAPTER-6

MATERIALS AND RESEARCH METHODOLOGY

1. **Synthesis of silver nanoparticles:**

   Synthesis of silver nanoparticles is done by green method using orange peel *Citrus sinensis* **(Balashanmugam et al., 2013)**

   - Orange peel were collected from fruit shop
   - Samples were taken to research lab
   - Samples were cleaned properly with distilled water
   - Samples were kept under aeration for complete drying for 3 to 4 days
   - Dried fruit peel were cut into small pieces and were powered
   - 2g of powered peel was added to 50ml of distilled water
   - It was boiled for 20 minutes at 50^{o} C
   - Crude extract were filtered using filter paper
   - Crude extract were stored in flask at 4^{o} C for further use
   - 1ml of extract was added to 9 ml of double distilled water making solution to10 ml in the test tube
   - Addition of 1mM of $AgNO_3$ to 10 ml filtrate in the test tube
   - Kept under dark conditions for 48 hours.
   - Reduction of silver ions from $AgNO_3$ to silver nanoparticles was monitored by spectroscopic methods at 420 nm.

2. Nanoparticle characterization by spectrophotometric method.
3. Activity of nanoparticles in solubilizing phosphate fertilizers- In this step we had taken two different P-fertilizers, then different amounts of fertilizers was treated with different concentrations of nanoparticles, like(Table no. 1.1)

10 g fertilizer	50 ml nanoparticles
	100 ml
	200 ml
25 g fertilizer	50 ml nanoparticles
	100 ml
	200 ml
50 g fertilizer	50 ml nanoparticles
	100 ml
	200 ml

Table 1.1

4. Then solubilization was checked by estimating phosphorus spectrophotometrically by molybdate reaction.
5. Then characterization of nanoparticles, P fertilizer before treatment and P fertilizer after treatment were done by TEM.
6. Then bioassay was performed with *Capsicum annum* a vegetable crop. In bioassay, application different P fertilizers to the crop along with the post potential amount of nanoparticles (identified from the above test) and estimation of available phosphorus according to the protocol enclosed from different plant parts at different stages of life cycle.

3.3.5.12 Estimation of phosphorus

The estimation of phosphorus content was done according to the method described by

Reagents:

1. **Tri acid mixture**: was prepared by mixing nitric acid, sulphuric acid and perchloric acid in the ratio 9:4:1.
2. **Ammonium molybdate reagent:** was prepared by dissolving 22.5 g ammonium molybdate in 400 ml DW. Then 1.25 g of ammonium vanadate was dissolved in 300 ml of boiling distilled water. Mix two solutions and add 250 ml of conc. HNO_3 and make the volume upto 1000 ml.**(Gerdal, 1928)**

3.3.5.12.1 Standard phosphate solution

Standard solution for phosphorus estimation was prepared by dissolving 0.2195 g of KH_2PO_4 in 1000 ml distilled water, resulting in 50 ppm phosphorus solution. After this different volumes of this standard solution viz. 0, 0.4, 0.8, 1.2, 1.6, 2.0 ml of stock solution to give 0, 1, 2, 3, 4, 5 ppm solutions. Then 4 ml of ammonium vanadate solution was added to each tube and volume was made up to 20 ml with distilled water. Then the color was allowed to develop and OD was recorded at 420 nm and the standard curve was plotted on a graph paper, which was used for calculation of phosphorus in unknown sample **(Gerdal, 1928).**

3.3.5.12.2 Estimation

1 g plant material is taken and 10 ml tri acid mixture was added to it. The sample was allowed to dissolve overnight. Then the samples were digested on a hot plate at 100°C till they become colorless. Then the residue was dissolved in 20 ml distilled water. From this 5 ml of each sample was taken in a test tube and 4 ml ammonium molybdate reagent was added and the volume was made up to 20 ml with distilled water. The color was allowed to develop and the OD was recorded at 420 nm. The concentration of phosphorus in the samples was determined from the standard curve. **(Gerdal, 1928)**

Materials

List of chemicals (Table no. 1.2)

NAME OF CHEMICALS	QUANTITY REQUIRED
Silver Nitrate	10 gram
Ammonium molybdate	50 gram
Ammonium vanadate	5 gram
Nitric acid	1200 ml
Sulphuric acid	400 ml
Perchloric acid	100 ml
Dipottasium hydrogen phosphate	1500 gram

Table 1.2

List of equipments (Table no. 1.3)

Spectrophotometer	Weighing machine
UV-Visible spectrophotometer	Centrifuge machine
Autoclave machine	Hot plate

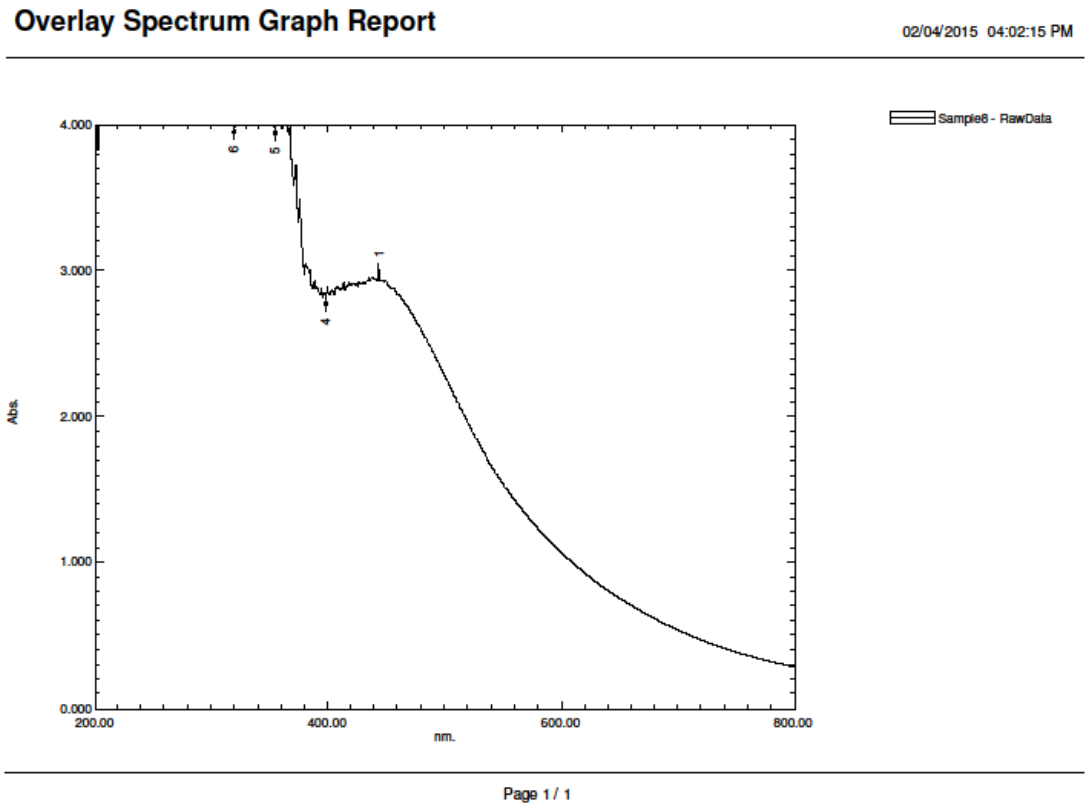
Table 1.3

CHAPTER - 7

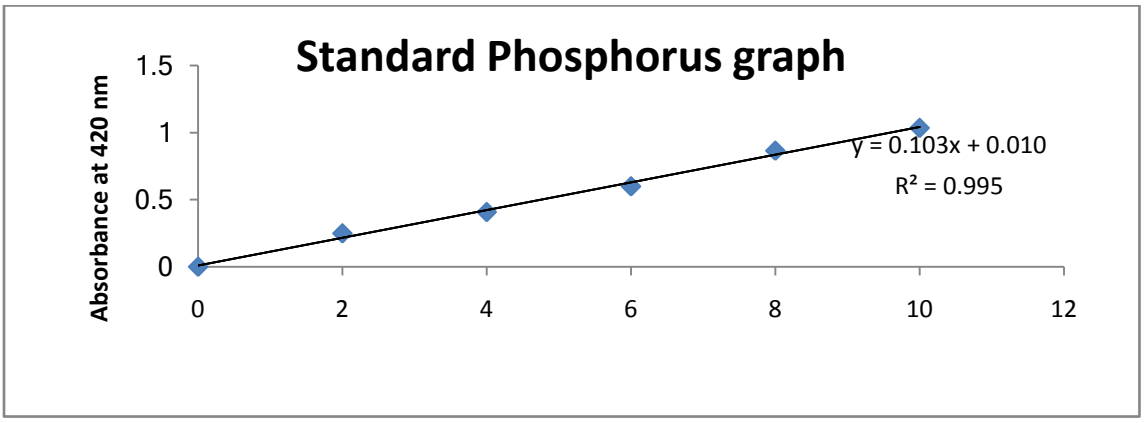
RESULT AND DISCUSSION

Result and Discussion

1. Silver Nanoparticles was obtained from the *Citrus sinesis* by following the standard protocol given by **Balashanmugam et al., 2013.** The graph showing the peak for silver nanoparticle at 435 nm. (Graph no. 1).



Graph no. 1.UV-Visible Spectrum of Orange Fruit peel Extract



Graph 2: Standard Phosphorus graph for the calculation of unknown conc. of Phosphorus in ppm (parts per million)

2. Optical density of different concentrations of Phosphorus fertilizer (10g, 25g, 50g) dissolved in different concentrations of nanoparticles (50ml, 100ml, 200ml) were taken after second , fourth sixth and eighth day of dissolution:

Concentration for 10 gram Phosphorus fertilizer **(Table 1.4)** given below

No. of day	Concentration (in ppm) at 50 ml	Concentration(in ppm) at 100 ml	Concentration(in ppm) at 200 ml
2nd day	0.067961	0.563107	0.019417
4th day	0.417476	0.087379	0.23301
6th day	0.407767	0.650485	0.563107
8th day	0.271845	0.262136	0.067961

Table 1.4 shows the concentration of 10 gram phosphorus fertilizer at two days interval. The minimum concentration at 50ml was observed in 2nd day i.e. (0.067961 ppm), at 100ml was observed in 4th day i.e (0.087379 ppm) and at 200 ml was observed in 2nd day i.e. (0.019417 ppm)

Concentration for 25 gram of Phosphorus fertilizer **(Table no.1.5)** given below

No. of day	Concentration(in ppm) at 50 ml	Concentration(inppm) at 100 ml	Concentration(in ppm) at 200 ml
2nd day	0.699029	0.300971	0.067961
4th day	0.058252	0.271845	0.330097
6th day	2.475728	0.398058	0.427184
8th day	1.601942	0.213592	0.165049

Table 1.5 shows the concentration of 25 gram phosphorus fertilizer at two days interval. The minimum concentration at 50ml was observed in 2nd day i.e. (0.699029), at 100ml was observed in 4th day i.e (0.165049) and at 200 ml was observed in 2nd day i.e. (0.067961)

Concentration for 50 gram of Phosphorus fertilizer: **(Table no. 1.6)**

No of day	Concentration(in ppm) at 50 ml	Concentration(in ppm) at 100 ml	Concentration (in ppm)at 200 ml
2nd day	1.563107	0.417476	0.135922
4th day	0.184466	0.223301	0.23301
6th day	1.242718	1.941748	0.184466
8th day	0.533981	0.271845	0.106796

Table 1.6 shows the concentration of 50 gram phosphorus fertilizer at two days interval. The minimum concentration at 50ml was observed in 2nd day i.e. (0.184466), at 100ml was observed in 4th day i.e. (0.21845) and at 200 ml was observed in 2nd day i.e. (0.106796)

Best concentration of standard Phosphate solution is 1.036. According to this, best concentration was selected from the O.D of different samples which was 0.265 (25 g of fertilizer in 50 ml of NPs solution on 6^{th} day). This best experimentally proved concentration was given to the 20 days old Chilli plant (6 chilli plants in which 4 plants were given the fertilizer and NPs solution and 1 plant having only fertilizer and I plant having no fertilizer NPs solution)

Concentration of plant species *(Capsicum annum)*: **(Table no. 1.7)**

No. of Samples	Concentration (in ppm)
Fertilizer + Nps	1.495146
Fertilizer + Nps	1.524272
Fertilizer + Nps	1.456311
Fertilizer + Nps	1.475728
Fertilizer	1.029126
No NPs , No Fertilizer	0.029126

It was observed that the addition of nanoparticles has increased the uptake of Phosphorus fertilizer. The Mean value of all four replicates of is 1.48786±0.02899 ppm. This value indicates that the nanoparticles have greatly influenced the uptake of phosphorus by plants. The phosphorus uptake of plant which is supplemented with only phosphorus fertilizer is showing the value of 1.029126 ppm while the plant which was grown in absence of fertilizer and nanoparticles is showing the least concentration which is 0.029126 ppm.

Experimental work

Synthesis of Silver Nanoparticles





Plants of *Capsicum annum* were grown

CHAPTER- 8

CONCLUSION AND FUTURE SCOPES

Conclusion

It was concluded that plant needs many nutrients for better growth and development. For adequate growth of plants some nutrients are required in large quantities while some are required in very low quantity. Fertilizers are of great importance to the plants which provides them sufficient nutrient. Phosphorus is one of them and plays very active role in the growth. The total phosphorus availability to the crop is very low and it must be changed to available forms by chemical process. The process is called as Phosphorus fixation. Nanoparticles have very efficient applications. They are used in medical, optical in many fields. Green synthesis of nanoparticles are very cost effective and the nanoparticles formed can be use to increase the bioavailability of phosphorus to the plants.

CHAPTER-9

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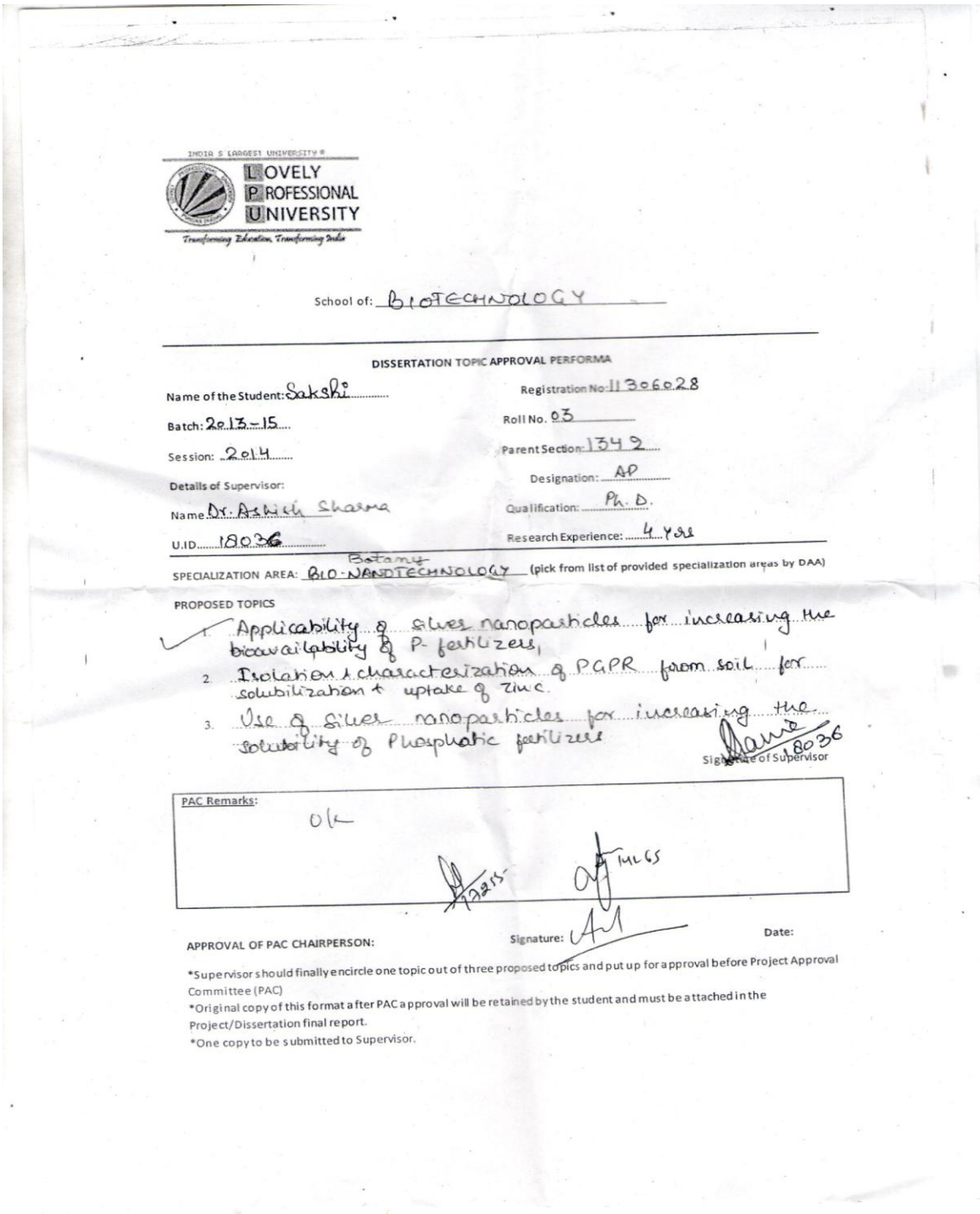
APPROVAL PAGE



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DISSERTATION TOPIC APPROVAL PERFORMA

Name of the Student: Sakshi Registration No: 11306028

Batch: 2013-15 Roll No. 03

Session: 2014 Parent Section: 1349

Details of Supervisor: Designation: AP

Name: Dr. Ashish Sharma Qualification: Ph. D.

U.ID: 18036 Research Experience: 4 yrs

SPECIALIZATION AREA: BIO-NANOTECHNOLOGY (Botany) (pick from list of provided specialization areas by DAA)

PROPOSED TOPICS

1. Applicability of Silver nanoparticles for increasing the bioavailability of P- fertilizers,
2. Isolation & characterization of PGPR from soil for solubilization & uptake of zinc.
3. Use of Silver nanoparticles for increasing the solubility of Phosphatic fertilizers

Signature of Supervisor 18036

PAC Remarks: Ok

APPROVAL OF PAC CHAIRPERSON: Signature: Date:

*Supervisor should finally encircle one topic out of three proposed topics and put up for approval before Project Approval Committee (PAC)

*Original copy of this format after PAC approval will be retained by the student and must be attached in the Project/Dissertation final report.

*One copy to be submitted to Supervisor.