EFFECT OF GREEN SYNTHESIZED SILVER NANOPARTICLES ON ANTIOXIDANT DEFENCE SYSTEM OF *Vigna radiata*

A thesis submitted to the Orissa University of Agriculture and Technology in partial fulfilment of the requirement for the degree of Master of Science in Biotechnology

By

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DECLARATION

I **Miss Madhusmita Jena, (Adm. No-05BT/17)** hereby declare that I am a candidate for the **Degree of Master of Science in Biotechnology, Department of Biotechnology, College of Basic Science And Humanities, Orissa University of Agriculture And Technology, Bhubaneswar-751003**, has studied during the academic session of 2017-19 and accompanied with the thesis work on "**Effect of green synthesized silver nanoparticles on Antioxidant defence system of *Vigna radiata***" submitted in the partial fulfilment for the requirements of award of the aforesaid degree is my original work done under the supervision and guidance of Dr. Kajari Das, Assistant Professor, Department of Biotechnology, CBSH, OUAT and that the thesis has not formed the basis of award of any degree, diploma, associate ship or any other similar titles before.

Place: Bhubaneswar

Date:

(Madhusmita Jena)

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CERTIFICATE-I

This is to certify that **Miss Madhusmita Jena(Adm No.- 05BT/17**), student of Department of Biotechnology, College of Basic Science and Humanities, Orissa University of Agriculture And Technology, Bhubaneswar-751003, a candidate for the award of the **Degree of Master in Science**, in Biotechnology has worked under my guidance and supervision during the academic session of 2018-19 and accompanying the thesis entitled "**Effect of green synthesized silver nano-particles on Antioxidant defence system of *Vigna radiata***" submitted in partial fulfilment for requirement of the award of the aforesaid degree, her genuine work. The assistance and help received by her during the course of investigation and sources of literature have been duly acknowledged.

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CERTIFICATE-II

This is to certify that the thesis "**Effect of green synthesized silver nano-particles on Antioxidant defence system of *Vigna radiata***" Submitted by Miss Madhusmita Jena, Adm No.- 05BT/17 to the Orissa University of Agriculture And Technology, Bhubaneswar-751003, in partial fulfilment of the requirement for the award of the degree of Master in Science in Biotechnology has been approved after an oral examination with the external examiners.

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ABSTRACT

The aim of this study was to find the effect of silver for enhanced plant growth, protein content and antioxidant enzyme activity of mungbean (*Vigna radiata*) under the influence of different concentrations of silver nanoparticles synthesised from teak leaves. The effect of various concentration of silver NP solution has insignificant effect on the plant morphology though substantial changes were observed in oxidative stress parameters in treated plants. As the root cells are exposed directly to the NP, the present investigation on biochemical parameters were accomplished in only roots of the treated plants. The protein concentration in root of the seedling was recorded to be enhanced with decrease in the concentration of silver NPs as compared to control which is also similar as peroxidase though at the lowest concentration SOD activity decreased significantly with increased protein and peroxidase. Chlorophyll content in all the treated plants were to be constant in comparison to control. With different concentration of NP exclusion, inclusion and accumulation in the treated plants are found to be changed significantly evidenced from ICP-AES.

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LIST OF SYMBOLS AND ABBREVIATION

%	Percentage
L	Litre
Ml	Mililitre
Mg	Milligram
Mm	Milimolar
Sec	Second
Min	Minute
Hr	Hour
Rpm	Revolution per minute
^0C	Degree celcius
Nm	Nanometre
EDTA	Ethylenediaminetetraacetic acid
DTT	Dithiothreitol
AgNP	Silver nanoparticle
NaOH	Sodium Hydroxide
$CuSO_4$	Copper Sulphate
$KNaC_4H_4O_6$	Sodium potassium tartarate
$FeCl_2$	Ferric chloride
$C_6H_6FeK_4N_6O_3$	Potassium ferricyanide

CHAPTER-1

INTRODUCTION

1.1 Background

Nanotechnology is the understanding & control of matter at dimensions of roughly 1 to 100 nm where unique phenomena enable novel applications. According to the National Nanotechnology Initiative (NNI), nanotechnology refers to the study of all particles have about 100 nanometers or less. The concept of nanotechnology emerged on 9th century. For the first time in 1959, Richard Feynman gave a talk on the concept of nanotechnology and described about molecular machines built with atomic precision where he discussed about nanoparticles and entitled that *"There's plenty of space at the bottom"* (T.C *et al*., 1974). The term "nanotechnology" first time used as scientific field by Nario Tanigushi in the 1974 his paper was "Nanotechnology" mainly consists of the processing of, separation, consolidation, and deformation of materials by one atom or one molecule (Zhang and Webster, 2008). At the nano scale, unique phenomena endow novel submissions. Latest developments in visualization and estimation systems for characterizing and checking components at the nano scale have led to a blast in nanotechnology-based components in areas such as polymers, plastics, electronics, vehicle constructing and surgery (Duncan, 2011). It is a rapidly growing industry that is expected to reach a market size of approximately 2.6 trillion dollars ($) by 2015 (Holman 2007). Nanoparticles have a size of one billionth of a meter (i.e. 10^{-9} m). Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution and morphology. The intrinsic properties of metal nanoparticles are mainly determined by size, shape, composition, crystallinity and morphology. As compared to the bulk material, nanoparticles show novel physical, chemical, electrical, mechanical, thermal, optical, dielectric and biological properties. Due to nano size, they exhibit extremely larger surface area in contrast with their volume, which lowers their melting temperature by hundreds of degrees than bulk material (Balaguru and Jeyaprakash, 2010). Conversion of metals into nanoparticles exhibits different striking colors due to the effect of Surface Plasmon Resonance (SPR) (Rivera *et al*., 2012). Thus in recent years much research is going on metallic nanoparticle and its properties like catalyst, sensing to optics, antibacterial activity, data storage capacity (Sharma *et al.,* 2009).

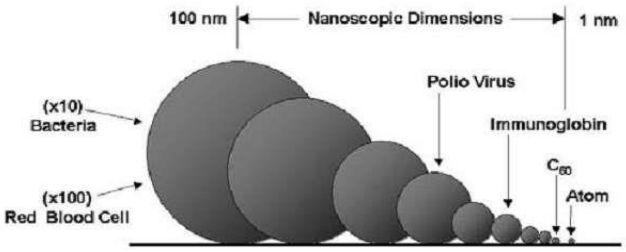

Fig 1- comparison of molecules at the nanometer scale

1.2 Silver Nanoparticles

Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes. In medicines, silver and silver nanoparticles have an ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices and implants prepared with silver-impregnated polymers. In textile industry, silver-embedded fabrics are now used in sporting equipment. Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. Nanoparticles can be linked to biological molecules that can act as address tags, to direct the nanoparticles to specific sites within the body (*Akerman et al.*), specific organelles within the cell (*Hoshino et al.*), or to follow specifically the movement of individual protein or RNA molecules in living cells (*Suzuki et al.*). Multivalent nanoparticles, bearing multiple targeting groups, can cluster receptors, which can activate cellular signaling pathways, and give stronger anchoring. Monovalent nanoparticles, bearing a single binding site, (*Sung et al., Fu et al., Howarth et al.*) avoid clustering and so are preferable for tracking the behavior of individual proteins.

1.2.2 Silver nanoparticles as an antimicrobial agent

Ag NP are highly antimicrobial to several species of bacteria, including the common kitchen microbe, *E. coli*. According to the mechanism reported, silver nanoparticles interact with the outer membrane of bacteria, and arrest the respiration and some other metabolic pathway that leads to the death of the bacteria. Silver is the metal of choice as they hold the promise to kill microbes effectively. Silver nanoparticles have been recently known to be a promising antimicrobial agent that acts on a broad range of target sites both extracellularly as well as intracellularly (Shrivastava et al.,2007), and also it was found to be in few studies (Zeng et al.,2007; Roe et al., 2008).

1.2.3 Synthesis of silver nanoparticles

Synthesis of nanoparticles having desired properties is one of the most challenging areas of research in nanotechnology. Researchers from various disciplines are engaged in exploring the best protocol for the synthesis of stable and monodispersed nanoparticles. Till today numerous techniques/methods for nanoparticles synthesis have been reported. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly byproducts along with high energy input and costly downstream processing. The need for environmental non-toxic synthetic protocols for nanoparticles synthesis leads to the developing interest in biological approaches which are free from the use of toxic chemicals as byproducts. Thus, there is an increasing demand for green nanotechnology. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals as well as provide natural capping agents and other advantages such as rapid synthesis, high yields & lack of costly downstream processing. Moreover, use of plant extracts also reduces the cost of microbes isolation and culture media enhancing the cost competitive feasibility over nanoparticles synthesis by microorganisms. There have been several experiments performed on the synthesis of silver nanoparticles using medicinal plants such as *Oryza sativa*, *Helianthus annus*, *Saccharum officinarum*, *Sorghum bicolour*, *Zea mays*, *Basella alba*, *Aloe vera Capsicum annuum*, *Magnolia kobus*, *Medicago sativa* (Alfalfa), Ci*namomum camphora* and *Geranium* sp. in the field of pharmaceutical applications and biological industries.

Teak leaf extract has been used as a reducing and stabilizing agent for the synthesis of AgNP's. *Tectona grandis* (Teak) is a large deciduous tree and an indigenous native of Asia, reputed as Sagwan (Hindi), Saka (Sanskrit) and teak tree (English). Its growing demand as the world's premium hardwood has initiated several countries lying in the tropical belt to grow them in huge plantations. Consequently, the proportion of foliage that forms the refuse of the timber industry is enormous, making it an ideal resource for the green synthesis of AgNps. Extracts obtained from teak leaves have demonstrated antioxidant activities, free radical scavenging properties (Rao *et al*), diuretic and antibacterial property (Arun *et al*). These "bioreservoirs" of active components go untapped as the timber obtained from these trees is used for lumbering while the leaves end up as agro-waste. The potency of these leaves can be consigned towards biosynthesis of nanoparticles. The precept of utilizing agro-waste resources for the synthesis of nanoparticles affirms to be green, self-sustaining and environmentally benign (David *et al*). In the present work, Teak leaves, an unharnessed agricultural waste, have been exploited as a resource for the effectual green synthesis of

nanoparticle under ambient conditions using the aqueous extract obtained from the leaves containing a multitude of bioactive compounds that act as reducing and capping agents for nanoparticles.

1.3 Effect of nanoparticles on antioxidant activity of *Vigna radiata*

The mung bean (*Vigna radiata*), is a plant species in the legume family. The mung bean is mainly cultivated in East Asia, Southeast Asia and Indian subcontinent. Importantly, mung beans are composed of about 20%–24% protein. Globulin and albumin are the main storage proteins found in mung bean seeds and make up over 60% and 25% of the total mung bean protein, respectively. In addition to high protein and low energy content, mung beans also contain various enzymes and plentiful microelements for example, superoxide dismutase (SOD). Mung beans have been shown to possess antioxidant, antimicrobial, and anti-inflammatory activities. As green gram is a stress sensitive legume as well as staple food of Odisha, the present investigation was undertaken to analyse whether some agent produces oxidative stress during seedling development.

1.3.1 Reactive Oxygen Species

The extracted nanoparticles were used to study the effect on antioxidant activity of roots of mung (*Vigna radiata*) seedlings. In the external environment, plants are under various abiotic and biotic stress. An important response to stress by aerobic cells is the production of reactive oxygen species (ROS), like superoxide radical (O2 .–), hydroxyl radical (.OH), alkoxy radical (.RO), singlet oxygen ($1O_2$), and toxic hydrogen peroxide (H_2O_2) molecules (Salin, 1988, Luna et al., 1994, Asada, 1999, Breusegem et al., 2001), which can cause oxidative damage to plants. These ROS produced in the cell are detoxified by both non enzymatic and enzymatic antioxidant system. ROS if not detoxified cause serious damage to proteins, lipids and nucleic acids (Elstner, 1984), Alscher et al., 1997). When the level of ROS is increased and exceeds the defense mechanism, the cell is in a oxidative stress. High concentrations of ROS are highly harmful, and when the symptoms persist, irreversible damage to the cells occurs, resulting in loss of physiological capacity and eventual cell death. Therefore, defense mechanisms against oxidative damage are activated during stress to regulate toxic levels of ROS.

1.3.2 Antioxidant Defense System

Plants have developed a complex antioxidant system against the reactive oxygen species generated in plant tissue during normal and stressful condition. These ROS produced in the cell are detoxified by both non enzymatic and enzymatic antioxidant system. Nonenzymatic antioxidants are found in all cellular components. These compounds may act directly in the detoxification of ROS and radicals, or they can reduce substrates for antioxidant enzymes. Nonenzymatic antioxidants include ascorbate (AsA) and glutathione (GSH) as well as tocopherol, carotenoids and phenolic compounds. Enzymatic components of the antioxidative defense system comprise several antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX, EC 1.11.1.9), guaiacol peroxidase (POX, EC 1.11.1.7), and peroxiredoxins (Prxs, EC 1.11.1.15), which catalyze ROS degradation, and enzymes of the ascorbate-glutathione (AsA-GSH) cycle, such as ascorbate peroxidase (APX, EC 1.1.11.1), monodehydroascorbate reductase (MDAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1), and glutathione reductase (GR, EC 1.8.1.7), that regenerate soluble antioxidants. This antioxidant system plays an important role in the maintenance of cell homeostasis and in the antioxidant response in plants.

OBJECTIVE

- To study the effect of silver nanoparticle as a plant growth promoting agent.
- Green synthesis of silver nanoparticles
    - Collection of plant material and preparation of aqueous leaf extract.
    - Synthesis followed by characterization of Ag nanoparticles.
- Study the effect of extracted nanoparticles on *Vigna radiata*
    - Germination of mung beans.
    - Inoculation of mung beans in Agar medium containing AgNPs.
    - To study the oxidative stress parameters (SOD, Pox, CAT).
    - To study the protein content & chlorophyll content in AgNP treated plants.
    - To study the morphological changes in the plants.

CHAPTER-2

REVIEW OF LITERATURE

Silver nanoparticles were formed by extracting *Eucalyptus hybrida* leaf. Bioactive
silver nanoparticle synthesis by reacting the methanolic biomass of *Eucalyptus hybrida* leaf with
aqueous solutions of silver nitrate. (Dubey *et al*, 2009)

Saxena *et al* (2010), used onion (*Allium cepa*) extract to synthesize silver nanoparticles at a rapid rate. In the research, silver nitrate in the aqueous was reduced by onion extract. It was concluded that onion extract increases the reaction rate and hence is a convenient method of synthesizing nanoparticles.

Elumalai *et al* (2010), used leaves of *Euphorbia hirta* to form silver nanoparticles. In the process dried leaves of the plant were mixed with aqueous solution of silver nitrate and stored at a low temperate. After centrifugation the supernatant was exposed to very high temperature forming silver nanoparticles.

The effect of nano-ZnO particles on growth of mung bean (*Vigna radiata*) and chickpea (*Cicer arietinum*) seedlings was studied. The study was conducted in plant agar media to prevent precipitation of water insoluble nanoparticles in test units. Various concentrations of nano-ZnO particles in suspension form were introduced to the agar media and its effect on root and shoot growth of seedlings examined. Inductive coupled plasma (ICP) was used to determine the uptake of nano-ZnO particles by root. The best response of nano-ZnO on mung bean was observed at 20 ppm and on chickpea at 1 ppm, beyond these concentrations the seedling showed retardation in growth and development thereby suggesting the toxic effect of nano-ZnO particles. (Mahajan *et al* 2011)

Nanoparticles were synthesized by using aqueous extract of *Moringa oleifera* and metal ions (silver ions). *M. oleifera* leaf extract was selected as it is of high medicinal value and it does not require any sample preparation and hence is cost-effective. The fixed ratio of plant extract and silver ions were mixed and kept at room temperature for reduction. The color change from yellow to reddish brown confirmed the formation of nanoparticles. Further, the synthesized nanoparticles were characterized by UV, EPMA, XRD and FTIR data. (Mubayi *et al*, 2012).

Hediat M. H. Salama, 2012 studied the effects of silver nanoparticles on plant growth parameters such as shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of economic important pulses, common bean (*Phaseolus vulgaris* L.) and corn (*Zea mays* L.). The study was carried out in a randomized block design with three replications. Five levels of silver nanoparticles (20, 40, 60, 80 and 100 ppm) were used. After germination, daily supply with 15 ml from each concentration was carried out for 12 days during plant growth. The results showed that small concentrations of silver nanoparticles had a stimulating effect on the growth of the plantlets, while the enhanced concentrations induced an inhibitory effect. However, increasing concentration of silver nanoparticles from 20 to 60 ppm has led to an increase in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of the two tested crop plants. Additionally, the lowest amount of these parameters was found with control plants, but the enhancing level of silver nanoparticles resulting in the reduction of these compounds.

Sharma *et al. 2012,* underlined the effect of silver metal nanoparticles (at 0, 25, 50, 100, 200 and 400 ppm) on the growth and antioxidant status of 7-day-old *Brassica juncea* seedlings. Fresh weight, root and shoot length, and vigor index of seedlings is positively affected by silver nanoparticle treatment. It induced a 326 % increase in root length and 133 % increase in vigor index of the treated seedlings. Improved photosynthetic quantum efficiency and higher chlorophyll contents were recorded in leaves of treated seedlings, as compared to the control seedlings. Levels of malondialdehyde and hydrogen peroxide decreased in the treated seedlings. Nanoparticle treatment induced the activities of specific antioxidant enzymes, resulting in reduced reactive oxygen species levels. Decrease in proline content confirmed the improvement in antioxidant status of the treated seedlings. The observed stimulatory effects of silver nanoparticles are found to be dose dependent, with 50 ppm treatment being optimum for eliciting growth response.

Okafor *et al*, (2013) used an environmentally friendly extracellular biosynthetic technique for the production of the AgNPs. The reducing agents used to produce the nanoparticles were from aqueous extracts made from the leaves of various plants. Synthesis of colloidal AgNPs was monitored by UV-Visible spectroscopy. The UV-Visible spectrum showed a peak between 417 and 425 nm corresponding to the Plasmon absorbance of the AgNPs. The characterization of the AgNPs such as their size and shape was performed by Atom Force Microscopy (AFM), and Transmission Electron Microscopy (TEM) techniques which indicated a size range of 3 to

15 nm. The anti-bacterial activity of AgNPs was investigated at concentrations between 2 and 15 ppm for Gram-negative and Gram-positive bacteria. Staphylococcus aureus and Kocuria rhizophila, Bacillus thuringiensis (Gram-positive organisms); *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* (Gram-negative organisms) were exposed to AgNPs using Bioscreen C. The results indicated that AgNPs at a concentration of 2 and 4 ppm, inhibited bacterial growth. Preliminary evaluation of cytotoxicity of biosynthesized silver nanoparticles was accomplished using the InQ™ Cell Research System instrument with HEK 293 cells. This investigation demonstrated that silver nanoparticles with a concentration of 2 ppm and 4 ppm were not toxic for human healthy cells, but inhibit bacterial growth.

Manivasagan *et al*. (2013) have reported the biological synthesis of silver nanoparticles using a novel actinobacteria species (*Nocardiopsis* sp. MBRC-1). Synthesized nanoparticles were spherical in shape with an average particle size of 45 ± 0.15 nm. These particles were bioactive in nature and demonstrated antimicrobial and cytotoxic activity in various in vitro assays.

Banerjee *et al*. Bioresources and Bioprocessing,2014 investigated an efficient and sustainable route of AgNP preparation from 1 mM aqueous AgNO3 using leaf extracts of three plants, *Musa balbisiana* (banana), *Azadirachta indica* (neem) and *Ocimum tenuiflorum* (black tulsi), well adorned for their wide availability and medicinal property. The AgNPs were characterized and tested for their antibacterial activity and toxicity. The AgNPs were characterized by UV-visible (vis) spectrophotometer, particle size analyzer (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy-dispersive spectroscopy (EDS). Fourier transform infrared spectrometer (FTIR) analysis was carried out to determine the nature of the capping agents in each of these leaf extracts. AgNPs obtained showed significantly higher antimicrobial activities against *Escherichia coli (E. coli)* and *Bacillus sp*. in comparison to both AgNO3 and raw plant extracts. Additionally, a toxicity evaluation of these AgNP containing solutions was carried out on seeds of Moong Bean (*Vigna radiata*) and Chickpea (*Cicer arietinum*). The seeds treated with AgNP solutions exhibited better rates of germination and oxidative stress enzyme activity nearing control levels, though detailed mechanism of uptake and translocation are yet to be analyzed. The AgNPs prepared are safe to be discharged in the environment and possibly utilized in processes of pollution remediation. AgNPs may also be efficiently utilized in agricultural research to obtain better health of crop plants.

Devadiga *et al*, 2015 used teak leaves, an agro -biowaste from world's premier hardwood timber industry, were used for ''green'' synthesis of silver nanoparticles (AgNPs). Bioactive compounds of leaves act as prolific reducing and stabilizing agents in AgNP synthesis. The characterization of the AgNPs synthesized using teak leaves revealed that the particles are spherical with an average size of 28 nm and the presence of bioactive compounds present in teak leaf extract as capping agents on the nanoparticles. A prominent decrease in the content of bioactive compounds such as polyphenols, antioxidants and flavonoids after the biosynthesis of AgNPs signifies that these class of compounds act as reductants and stabilizers during biosynthesis. The biosynthesized silver nanoparticles were also successfully evaluated for their antibacterial characteristics against waterborne pathogens, *E. coli* and *S. aureus*, with minimum inhibitory concentration of 25.6 lg/mL. Exploitation of agrowaste resources for synthesis of AgNPs curtails indiscriminate usage of food and commercial plant materials, rather contributing a sustainable way for effective plant waste biomass utilization and management. The biosynthesized AgNps have potential application in water purifiers, antibacterial fabrics, sports wear and in cosmetics as antibacterial agent and the process used for its synthesis being greener is highly beneficial from environmental, energy consumption and economic perspectives.

Raliya *et al. 2015* synthesiszed TiO_2 NP using the fungi *Aspergillus flavus* TFR 7 and evaluated their influence on mung bean. The characterized TiO_2 NPs were foliar sprayed at 10 mgL^{-1} concentration on the leaves of 14 days old mung bean plants. A significant improvement was observed in shoot length (17.02%), root length (49.6%), root area (43%), root nodule(67.5%), chlorophyll content (46.4%) and total soluble leaf protein (94%) as a result of TiO_2 NPs application. In the rhizosphere microbial population increased by 21.4–48.1% and activity of acid phosphatase (67.3%), alkaline phosphatase (72%), phytase (64%) and dehydrogenase (108.7%) enzyme was observed over control in six weeks old plants owing to application of TiO_2 NPs. A possible mechanism has also been hypothesized for TiO_2 NPs biosynthesis.

Arabaci *et al*, 2016 measured the antioxidant enzyme (polyphenol oxidase, peroxidase, catalase and superoxide dismutase) activities of crude extract of *Rumex obtusifolius* L. in order to gain insight about this plant's antioxidant potential. Enzymatic antioxidant activity of this plant was investigated by carrying out catalase, superoxide dismutase, peroxidase and polyphenol

oxidase enzyme activity assays. Enzyme activities of the crude extract were measured by using spectrophotometric method. Optimum pH and temperature values of each enzyme were also determined for measurement of enzyme activities in ideal conditions. The results showed that *Rumex obtusifolius* L. crude extract had good activity for all the enzymatic procedures tested. The activity levels of enzymatic antioxidants polyphenol oxidase, peroxidase, catalase and superoxide dismutase of the plant were found to be 12.8; 195.2; 238.7; 11.6 EU/mL, respectively. Optimum pH and temperature values of all the enzymes (except PPO: optimum temperature 30°C) tested were also found to be 7.0 and 25°C, respectively. Results demonstrated that this edible plant, *Rumex obtusifolius* L., might be a potential source of natural antioxidants with good antioxidant enzyme capacity.

Pallavi et al, 2016 investigated the impact of silver nanoparticles (AgNPs) on the growth of three different crop species, wheat (*Triticum aestivum*, var. UP2338), cowpea (*Vigna sinensis*, var. Pusa Komal), and Brassica (*Brassica juncea*, var. Pusa Jai Kisan), along with their impact on the rhizospheric bacterial diversity. Three different concentrations (0, 50 and 75 ppm) of AgNPs were applied through foliar spray. After harvesting, shoot and root parameters were compared, and it was observed that wheat was relatively unaffected by all AgNP treatments. The optimum growth promotion and increased root nodulation were observed at 50 ppm treatment in cowpea, while improved shoot parameters were recorded at 75 ppm in Brassica. To observe the impact of AgNPs on soil bacterial community, sampling was carried out from the rhizosphere of these crops at 20 and 40 days after the spraying of AgNPS. The bacterial diversity of these samples was analyzed by both cultural and molecular techniques (denaturing gradient gel electrophoresis). It is clearly evident from the results that application of AgNPs changes the soil bacterial diversity and this is further influenced by the plant species grown in that soil. Also, the functional bacterial diversity differed with different concentrations of AgNPs.

Zuverza-Mena *et al*, 2016 studied the impacts of nAg on the physiology and nutritional quality of radish (*Raphanus sativus*) sprouts. Seeds were germinated and grown for 5 days in nAg suspensions at 0, 125, 250, and 500 mg/L. Seed germination and seedling growth were evaluated with traditional methodologies; the uptake of Ag and nutrients was quantified by inductively coupled plasma-optical emission spectroscopy (ICP-OES) and changes in macromolecules were analyzed by infrared (IR) spectroscopy. None of the nAg concentrations

reduced seed germination. However, the water content (% of the total weight) was reduced by 1.62, 1.65, and 2.54% with exposure to 125, 250, and 500 mg/L, respectively, compared with the control. At 500 mg/L, the root and shoot lengths were reduced by 47.7 and 40%, with respect to the control. The seedlings exposed to 500 mg/L had 901 ± 150 mg Ag/kg dry wt and significantly less Ca, Mg, B, Cu, Mn, and Zn, compared with the control. The infrared spectroscopy analysis showed changes in the bands corresponding to lipids (3000–2800 cm^{-1}), proteins (1550–1530 cm^{-1}), and structural components of plant cells such as lignin, pectin, and cellulose. These results suggest that nAg could significantly affect the growth, nutrient content and macromolecule conformation in radish sprouts, with unknown consequences for human health.

Olchowik *et al*, 2017 evaluated the effect of silver (AgNPs) and copper nanoparticles (CuNPs) on the growth parameters, on the extent of leaves infected by powdery mildew and on spontaneous ectomycorrhizal colonization of English oak (Quercus robur L.) seedlings growing in containers. Nanoparticles were applied to foliage four times during one vegetation season, at four concentrations: 0, 5, 25 and 50 ppm. The adsorption of NPs to leaves was observed by microscopical imaging (TEM). The tested concentrations of AgNPs and CuNPs did not have any significant effect on the growth parameters of the oak seedlings. TEM results showed disturbances in the shape of plastids, plastoglobules and the starch content of oak leaves treated with 50 ppm Cu- and AgNPs, while no changes in the ultrastructure of stems and roots of oak plants treated with NPs were observed. No significant difference in powdery mildew disease intensity was observed after NP foliar application. Four ectomycorrhizal taxa were detected on oak roots (*Sphaerosporella brunnea, Thelephora terrestris, Paxillus involutus and Laccaria proxima*). Oak seedlings treated (foliar) with CuNPs and AgNPs at 25 ppm were characterised by the highest degree of mycorrhization (respectively, 37.1% and 37.5%) among all treatments including the control treatment. None of the tested NPs manifested phytotoxicity in the examined Q. robur seedlings under container nursery conditions.

Miri *et al*, 2017 investigated the effects of nickel nanoparticles on root and shoot elongation, relative water content (RWC), photosynthetic pigment, total ash, antioxidant activity of *Coriandrum sativum* L. The results showed that nickel nanoparticle decreased the RWC, root and shoot elongation, the content of photosynthetic pigments, %total ash. In addition, although

the nanoparticles decreased the antioxidant activity. The results of this study have shown that, nickel nanoparticles have toxic effects on *C. sativum* L plant.

Green synthesis of Ag, Cu and AgCu nanoparticles at room temperature using palm leaves extract was carried out. The purpose of this study was to eliminate the use of chemicals in the synthesis of nanoparticles and evaluate the efficiency of the palm leaves extract as the reducing and stabilizing agents. The palm leaves extract was added to metal salt solution and continuously stirred until reaction completed. The produced nanoparticles were analyzed using atomic absorption spectroscopy (AAS), Fourier transform infrared spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDS), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The analyses revealed that palm leaves extract has efficiently reduced the silver ions, but not the copper ions. During synthesis of AgCu nanoparticles, simultaneous reduction was occurred leading to formation of alloyed nanoparticles. Biomolecules from the palm leaves extract adsorbed on the surface of nanoparticles forming a capping layer thus stabilized the nanoparticles. The produced Ag and Cu nanoparticles were predominantly spherical with the particle size of Cu nanoparticles were larger than Ag nanoparticles. The AgCu nanoparticles closely resembled the Ag nanoparticles due to high Ag content with average size of 13nm. Therefore, palm leaves extract has a potential to be a good reducing and stabilizing agents. (Mohamad *et al*,2017)

T. grandis was used as a reducing agent in the synthesis of the AgNPs. The NPs were synthesized and characterized using different techniques such as ultraviolet-visible spectroscopy, Dynamic light scattering (DLS), Transmission electron microscopy (TEM), and Scanning electron microscopy (SEM). The synthesized NPs were then evaluated for their antimicrobial efficacy against the Gram-negative and Gram-positive bacteria and antifungal activity. Further, *in vitro* antioxidant efficacy of the AgNPs was calculated using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid and 2,2-diphenyl-1-picrylhydrazyl assay. From the above analyses, the formation of spherical NPs with an average size of 100 nm was confirmed. Minimum inhibitory concentration (MIC) and minimal biocidal concentration (MBC) of the AgNPs were calculated, MIC and MBC values ranged from 0.50 to 1.8 μg/mL and 0.91 to 3.6 μg/mL, respectively. The Aprepared NPs were found to be uniform in size with smooth topography. The antibacterial and antifungal efficacy of the NPs was found to be effective on the broad spectrum of microbes. The antioxidant activity of AgNPs was comparable to ascorbic acid. (Pinki Dangi & Jangir Op, 2019)

Rautela *et al* (2019) green synthesized silver nanoparticles from *Tectona grandis* seeds extract. The present study for the first time utilized seed extract of *Tectona grandis* (teak) for reduction of 1 mM silver nitrate solution to silver nanoparticles. Synthesis of nanoparticles was confirmed by visual detection in which the colorless solution gets changed to a brown-colored solution. Further characterization was done by UV-visible spectroscopy, XRD, FTIR analysis, SEM/EDS, FESEM, and TEM. Size of silver nanoparticles was found to be 10–30 nm approximately as determined by transmission electron microscopy (TEM). Energy-dispersive spectra (EDS) revealed that nanoparticles contain silver in its pure form. Well diffusion method showed the antimicrobial effect of AgNPs on different microorganisms with the zone of inhibition of 16 mm for Staphylococcus aureus, 12 mm for Bacillus cereus, and 17 mm for E. coli when 50 μg of AgNPs was used. Minimum inhibitory concentration was found to be 5.2, 2.6, and 2.0 μg/ml for Bacillus cereus, Staphylococcus aureus, and E. coli respectively. Mode of action of antimicrobial activity of nanoparticles was investigated by determining leakage of reducing sugars and proteins, suggesting that AgNPs were able to destroy membrane permeability.

Chapter 3

MATERIALS & METHODS

4.1 List of Instruments Used

INSTRUMENTS	COMPANY
Centrifuge	REMI CM12
Fridge	SAMSUNG
Magnetic stirrer	REMI
Laminar air flow	LABSOL
UV -Vis Spectrophotometer	SYSTRONICS
pH meter	ELICO LI 617
Autoclave	SHIVA
Vortex	SPINIX
Weighing Balance	WENSAR
Microscope	OLYMPUS CH20 I
Incubator	SMITA SCIENTIFIC
Hot Air oven	LABOTECH
Microwave	SAMSUNG
Pipette	FINNPIPETTE
Gel apparatus	BIORAD

4.2 Experimental Site:

The present experiment was conducted at the Department of Biotechnology, College of Basic Science and Humanities, Orissa University of Agriculture And Technology,Bhubaneswar-751003.

4.3 Media And Reagents Used:

All chemicals used were of analytical grade. Media and chemicals used in this study were purchased from Himedia, India & Sigma Aldrich, America.

4.4 Green synthesis of silver nanoparticles

4.4.1 Collection of plant material

Teak leaves were used for synthesis of silver nanoparticles. Firstly fresh leaves were collected from the backyard of College of Basic Science & Humanities, Bhubaneswar. Then the leaves were washed thoroughly in running water followed by double distilled water to remove debris and other surface contaminants. The midrib portion of the leaves were removed and then the leaves were properly wrapped in paper and shade dried for 5-7days. The leaves were crushed to fine powder using a mixer and their dry weight was taken and stored in amber colored dry containers for further use.

4.4.2 Preparation of aqueous teak leaf extract

The aqueous extract of teak leaves was prepared by adding 5g of processed teak leaf powder to 100ml pf distilled water in a conical flask and the leaf suspension was boiled for 5minutes at 100˚C. The suspension was further allowed to boil for 5minutes with continuous stirring. The suspension was then cooled to a temperature of 28±2°C. The crude extract, thus obtained, was filtered using Whatman No. 1 filter paper (Whatman Plc, Kent, UK) to eliminate any plant material. The filtrate was stored in amber coloured air tight bottles at 4°.

4.4.3 Synthesis of AgNP's

In a typical one-step synthesis, AgNPs were prepared by the reduction of AgNO3. To 75ml of sterile distilled water 20 ml of leaf extract and 5ml of 1mM silver nitrate solution was added. The solution was kept as such in dark for 24 hrs. The colour of the solution changed from pale yellow to amber colour confirming the synthesis of nanoparticle.

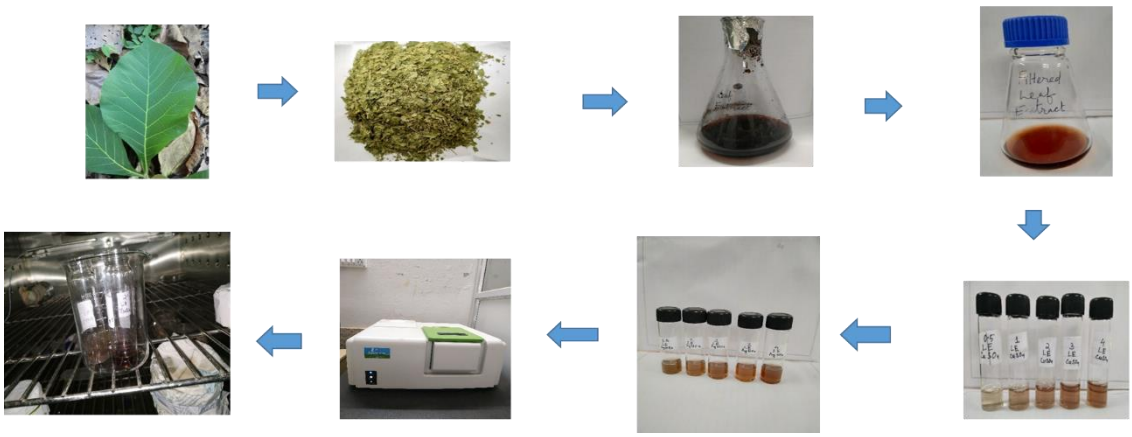

Fig2: green synthesis of silver nanoparticles

4.5 Characterization of silver nanoparticles

The technical application of nanoparticles mainly depends on their surface. It is therefore crucial to the chemist to control the surface and thus the properties of single particles. However, the qualitative and quantitative analysis of the surface of a single nanoparticle or a nanoparticle ensemble is challenging (Borm et al., 2006). The following methods are employed to characterize the nanoparticles:

4.5.1 UV-Vis spectroscopy :

The electronic structures of atoms, ions, molecules or crystals through exciting electrons from the ground to excited states (absorption) and relaxing from the excited to ground states (emission) are used for determination in UV-Vis spectroscopy. The metallic nanoparticles are also known to exhibit different characteristic color. These resonances are known as surface plasmons, which occur only in the case of nanoparticles and not in the case of bulk metallic particles (Papavassiliou, 1979). Noble metals are known to exhibit unique optical properties due to the property of surface plasmon resonance (SPR). The formation of silver nanoparticles was monitored with color change and UV Vis spectroscopy. The color of the reaction mixture started changing to yellowish brown within 45 min and to dark brown after 1 h, indicating the generation of silver nanoparticles, due to the reduction of silver metal ions into silver nanoparticles via the active molecules present in the leaf extract. This color is attributed to the excitation of SP. The UV-Visible spectra of silver nanoparticles were recorded as a function of wavelength using UV-Vis spectrophotometer operated at a resolution of 435nm. Scanning UV -Visible spectrophotometer (Perkin Elmer UV-Vis Lambda 365) with a scanning range

between 200-1000nm was used to observe colour change due to the surface plasmon resonance of the metallic nanoparticles. 1ml of the nanoparticle sample were pipetted into the cuvette and subsequently analysed at room temperature.

4.5.2 Dynamic Light Scattering (DLS) Analysis:

The shape and size of silver nanoparticles were determined by DLS analysis. Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. In order to determine the representative value of particle population and particle size distribution ,particle size analyser was used which measures the average particle size (Z value) by taking harmonic mean cumultants of scattered intensity**:**diameter of every single particle at 90^{o} scattering angle using Dynamic Light Scattering. The samples were further analysed at Institute of Life Science, Bhubaneswar.

4.5.3 Inductively coupled plasma atomic emission spectroscopy:

(ICP-AES), also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of chemical elements. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element. It is a flame technique with a flame temperature in a range from 6000 to 10,000 K. The intensity of this emission is indicative of the concentration of the element within the sample.

The plant sample was digested according to the following method:

To the sample 9ml of freshly prepared acid mixture of 65% HNO_3 and 37% HCl was added. Then, the mixture was boiled gently over a water bath ($95^{o}C$) for 4-5 hours (or until the sample had completely dissolved). (Ang and Lee 2005).During the digestion procedures, the inner walls of the beakers were washed with 2 mL of deionized water to prevent the loss of the sample, and at the last part of the digestion processes, the samples were filtered with Whatman Filter Paper No. 41 filter paper. The digested sample was analyzed at Central Laboratory, Orissa University of Agriculture And Technology, BBSR.

4.6 Germination of mung beans:

Seeds of plant species mung (*Vigna radiata*) were purchased locally. Seeds were kept in dry place in dark under room temperature prior to use. Mung gram seeds were washed in distilled water 2-3times and then sterilized in 4% sodium hypochlorite solution for 5min to ensure surface sterility and rinsed in distilled water several times. Mung seeds were allowed to germinate in wet absorbant cotton at controlled temp of 25± 1°C in dark for 24hrs. The seeds were checked for germination and sprouted seeds were used for further study.

4.7 Inoculation by plant agar method

1% plant tissue culture agar was prepared and properly sterilized in an autoclave and allowed to cool down to room temp. Varying concentration of Ag nanoparticles i.e.; 50, 100, 200 ppm was added to the sterilized glass tubes in replicates of 6 for each concentration. 10ml of 1% agar was added to each tubes and vortexed for proper mixing of nanoparticles in the Agar medium. In each tube 2 germinated plant seedlings were gently placed above the surface of agar media. Agar media without Ag nanoparticles were used as control. The tubes were incubated in controlled conditions of 25±1°C in dark and in light for 6-8hours. The seeds were left to grow and the results were observed and biochemical assay done after 7 days.

4.8 Biochemical Assays:

Different biochemical assays were carried out to observe the effect of nanoparticles on the roots of mung seedlings.

- **Preparation of enzyme extract:**
  The tissue for enzyme is freshly harvested. For preparing the extract, the sample was homogenized(10%) in ice cold 50mM phosphate buffer (pH=7.4) containing 1 mM EDTA and 1mM DTT. The homogenate was centrifuged at 10,000 g for 5 minutes at 4 °C. The supernatant was dispensed in aliquots and stored at -80 °C till further analysis.

4.8.1 PROTEIN ESTIMATION:

Protein estimation of samples were made according to Lowry et al (1951). Protein reagent was prepared by adding alkaline NaOH, $KNaC_4H_4O_6$ and $CuSO_4$ in the ratio 50:1:1. After 10 minutes of incubation 0.1ml of folin ciocalteau's reagent(1 part of commercially available reagent diluted with 2parts of distilled water) was added and

vortexed. After 30 minutes of incubation at room temperature absorbance was measured at 700nm by spectrophotometer against an appropriate blank. Protein content was expressed as mg/g wet weight of the tissue and aqueous BSA(bovine serum albumin)was taken as standard protein.

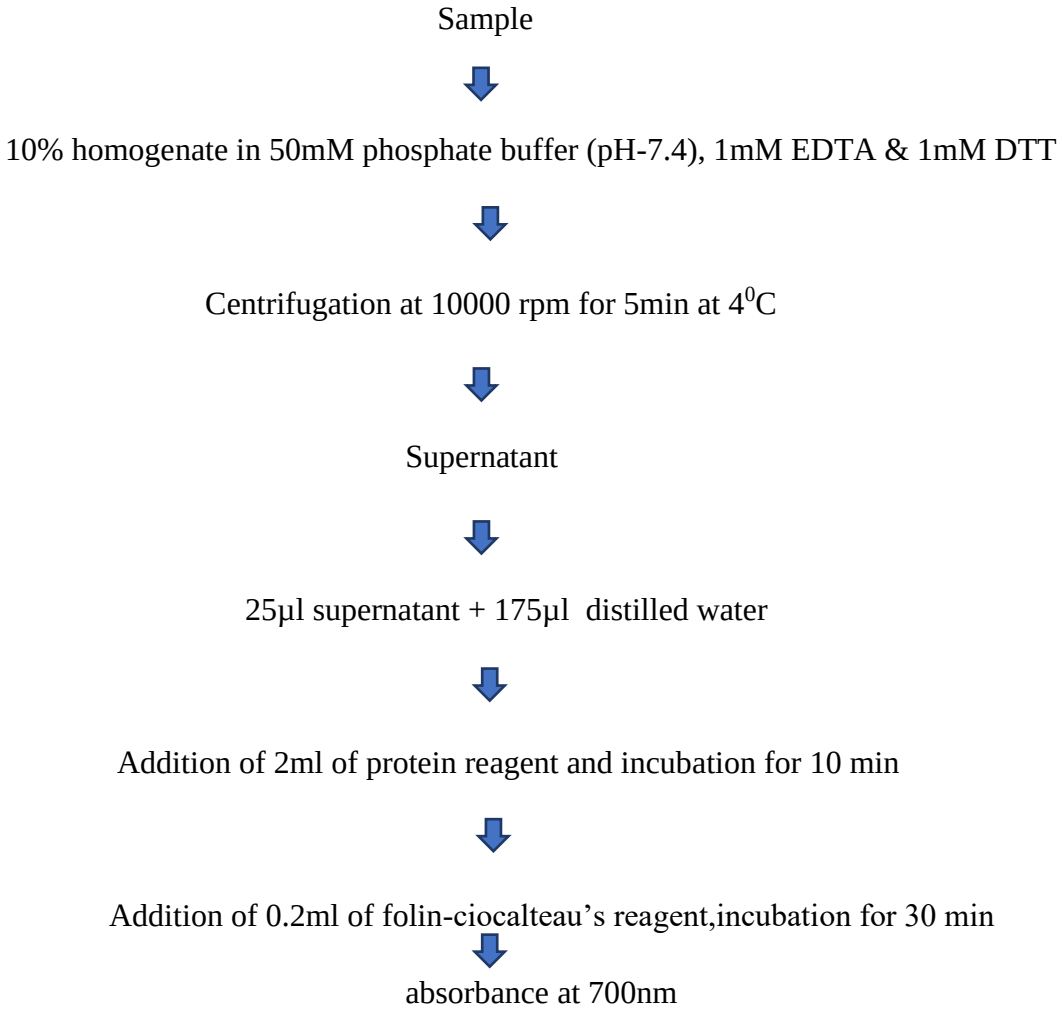

Fig 3: flow chart for protein estimation

4.8.2 ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)ACTIVITY

SOD(SOD;EC1.15.1.1)activity was determined according to the method of Das et al.(2000). In this method superoxide radicals are generated by photo reduction of riboflavin. Superoxide radicals were allowed to react with hydroxylamine hydrochloride to produce nitrite in turn reacts with sulphanilic acid to produce a diazonium compound,which subsequently reacts with N-1 Napthyl Ethylene Diamine Dihydrochloride (NED)to produce a red azo compound having absorption maxima at 543nm.Superoxide dismutase scavenger superoxide radicals produced

by photoreduction of riboflavin .Therefore, nitrite formation in the reaction is inversely proportional to the amount of SOD present in the sample.in this method 1.4ml aliquot of the cocktail was taken in a test tube to which ,0.1ml test sample was added followed by 80ul of riboflavin. The tubes were exposed for 10min to two 20W Philips CFL lamps fitted in an aluminium foil coated wooden box. At the end of the exposure time 1ml of Greiss reagent (prepared freshly by mixing equal volume of 1% sulphanilamide in 5% phosphoric acid and 0.1% NED)was added to the tube and the absorbance of the colour formed was measured at 543nm.One unit of enzyme activity is defined as the amount of SOD capable of inhibiting 50% of nitrite formation under assay condition. The enzyme activity is calculated from the value where $v_0$is the absorbance of the sample. SOD activity was expressed as units/mg protein.

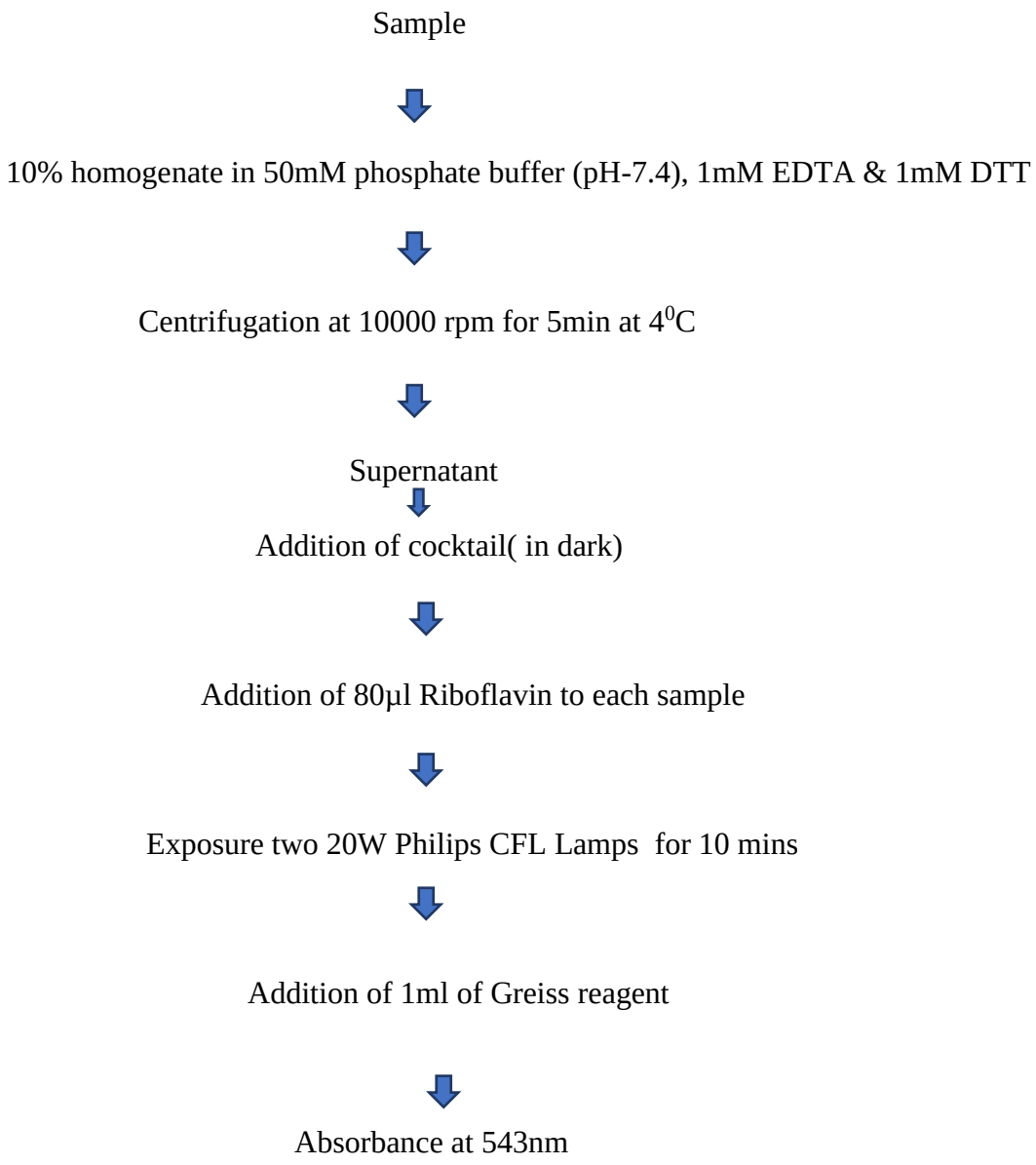

Fig 4: Flow chart of SOD estimation

4.8.3 ESTIMATION OF GUAICOL PEROXIDASE ACTIVITY:

Peroxidase (POD) (EC: 1.11.1.7) activity was measure by method of Chance and Maehly (1955). Peroxidase also referred as non-specific peroxidase or guaiacol-peroxidase catalyses the reduction of hydrogen peroxide with a concurrent oxidation of guaiacol to a colored tetraguaiacol. The increase in absorbance is recorded at 470 nm.

Assay solutions

Solution A: Sodium phosphate buffer (100 mM, pH 7.0)

Solution B: 10 mM H_2O_2 solution

Solution C: 20 mM guaiacol

The reaction was started by addition of solution C (20 mM guaiacol). The increase in absorbance was recorded for 2 min at 20 sec interval at 470 nm. The reaction mix without enzyme serves as blank. Enzyme activity is calculated as per extinction coefficient of its oxidation product, tetraguaiacol 25 mM-1 cm-1. Enzyme activity is expressed as µmol tetra-guaiacol formed per

min per mg protein.

4.8.4 CATALASE ACTIVITY STAIN

Non-denaturing page were conducted. After electrophoresis the protein were detected via the staining of the gel with coomassie's brilliant blue. Catalase activity on the non-denaturating polyacrylamide gels was visualized via ferric chloride potassium ferric cyanide double staining method, the gels were initially incubated with 5mm hydrogen peroxide. Followed by staining with a freshly prepared mixture of 2% ferric chloride and 2% potassium ferric cyanide (Yoon-Sukkang, 2006).

Enzyme extract

↓

7.5% Native Gel

↓

Electrophoresis at 100V for 2-3hours

↓

Rinsing gel with with distilled water

↓

Soaking in 50µl H_2O_2 + 20ml distilled water for 5min

↓

10ml 2% $FeCl_2$+ $[Fe(CN)_6]^{4-}$

↓

Washing with water immediately

↓

Dark blue background with clear catalase

Fig 5: Flow chart of catalase gel activity

4.8.5 ESTIMATION OF CHLOROPHYLL CONTENT:

Chlorophyll content was estimated according to Arnon's method. Fresh leaves were taken and 2-3ml of 80% acetone was added. The chlorophyll by thoroughly grinding it in a pesto-mortar. The sample was centrifuged at 3000 *g* for 15min. The absorbance for photosynthetic pigments at 663 and 645nm for chlorophyll a & b respectively as soon as possible as chlorophyll starts to disintegrate.

Leaves are grinded with Phosphate buffer

↓

Transferred to test tube

↓

Addition of 2-3ml of 80% acetone

↓

Incubation for 15min (in dark)

↓

Centrifugation at 3000rpm for 15min

↓

Supernatant is taken & diluted with 80% acetone (1:4)

↓

Absorbance is taken at 663nm & 645nm

Fig 6: Flow chart of chlorophyll estimation

4.8.6 MORPHOLOGICAL ANALYSIS

Morphological analysis was done by comparing the measurements of root, stem and leaf length of the AgNP treated plants with the non-treated plant.

CHAPTER-4

RESULTS AND DISCUSSION

RESULTS

4.1 Visual observation of AgNP synthesis

According to literature studies AgNP solution has dark brown colour. The color of *Tectona grandis* leaf extract was reddish brown but after its treatment with 1mM $AgNO_3$, its colour changes to yellowish brown. After further for incubation for 24hours the colour changed to dark brown due to reduction of silver metal ions into AgNPs via the active molecules present in the leaf extract which indicated the formation of AgNPs. This colour change is due to the property of quantum confinement which is a size dependent property of nanoparticles which affects the optical property of the nanoparticles.

Fig 7: color change indicating the synthesis of silver nanoparticles

4.2 Characterization by UV -Vis spectroscopy

The formation of AgNPs was monitored with colour change and UV-Vis spectroscopy. UV-Vis spectra shows the peak approximately at 420 nm clearly indicating the formation of AgNPs in leaf extract. The occurrence of peak at 420nm is due to the phenomenon of surface plasmon resonance which occurs due to the excitation of the surface plasmons present on the outer surface of the AgNPs which gets excited due to the applied electromagnetic field. Absorption peaks intensity increases according to time and the colour intensity increases with the duration of incubation.

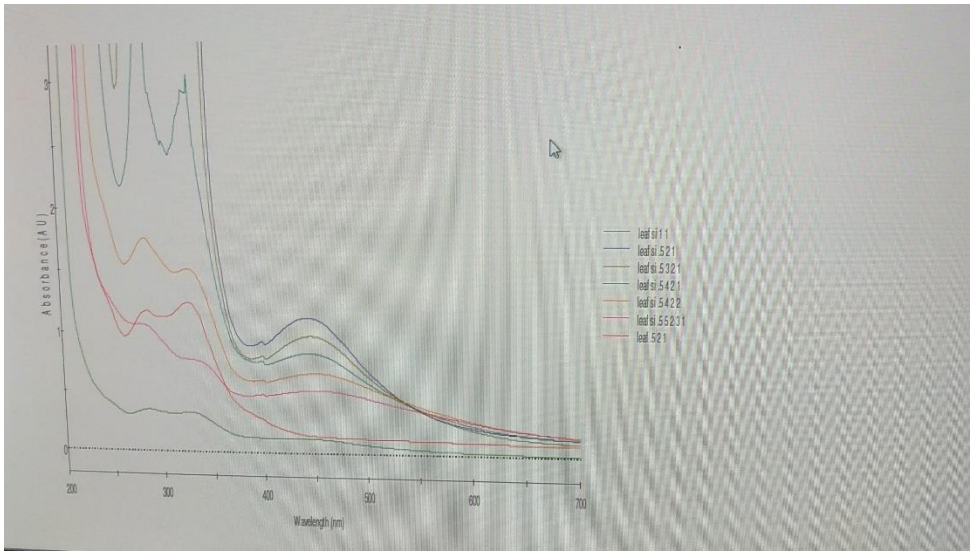

Fig 8: UV-Vis spectral analysis of AgNP synthesized by teak leaf

4.3 DLS analysis

The DLS size distribution image of biosynthesized of CuNPs is shown in fig.. The average particle size of AgNPs range from 10-1000nm.whereas the high intensity peak observed at 102nm. PDI value indicates that the AgNP were not aggregated.

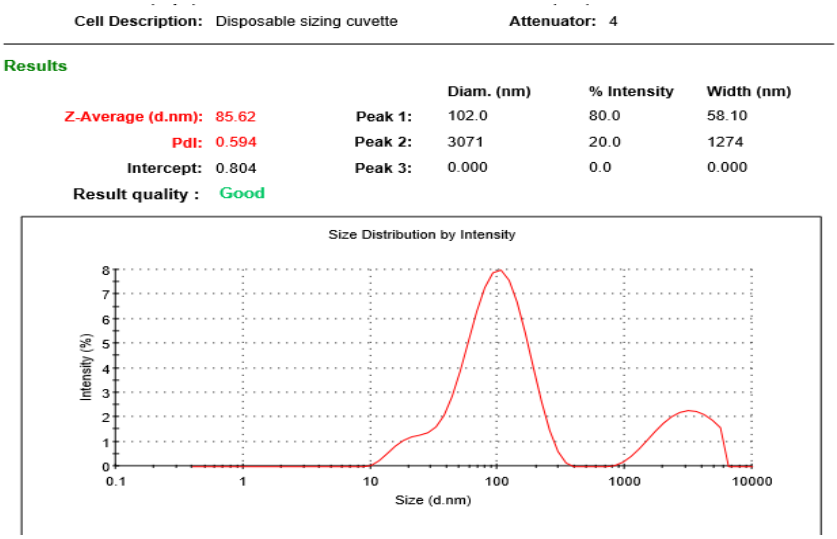

Fig9: DLS of synthesized AgNPs

4.4 Estimation of protein

The protein estimation was done by Lowry's method. The protein content (mg) per gram tissue was found to be highest in plant with lowest concentration of AgNP. All the AgNP treated plants were observed to have higher protein concentration than the non-treated plants.

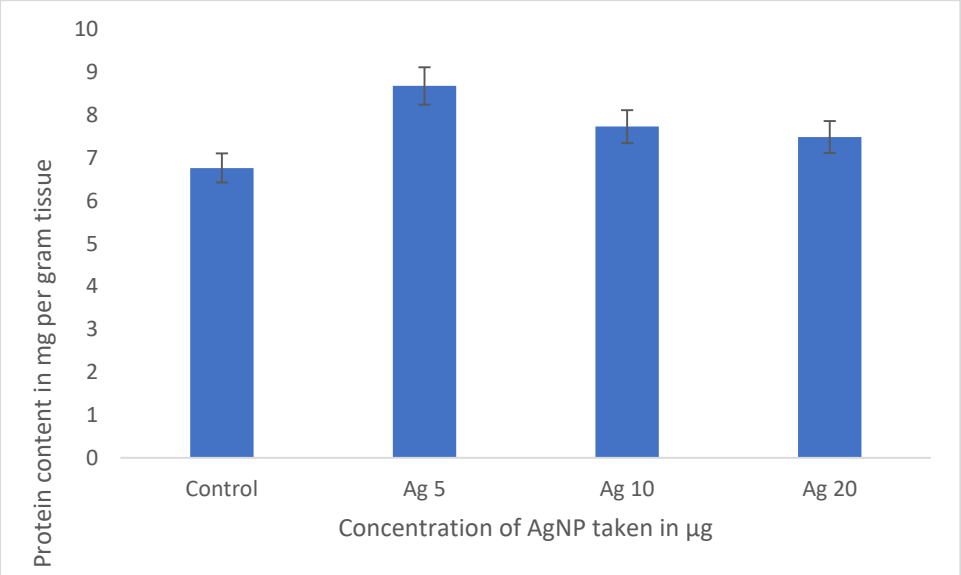

Fig10: Protein estimation of AgNP treated plants

4.5 Estimation of SOD Activity

Oxidative stress usually stimulates the activities of antioxidant enzymes, namely, SOD, peroxidase, and catalase. SOD operates at the early stages of oxidative reactions, converting the superoxide radical into hydrogen peroxide. In our experiments, SOD activity in the variant with nanoparticles was seen increasing as in maximum concentration of AgNP (200ppm). The pattern of changes in the activities of antioxidant enzymes (SOD, and peroxidase) differed significantly between experimental variants. In the variant with nanoparticles, SOD activity was lower in lowest concentration of AgNP.

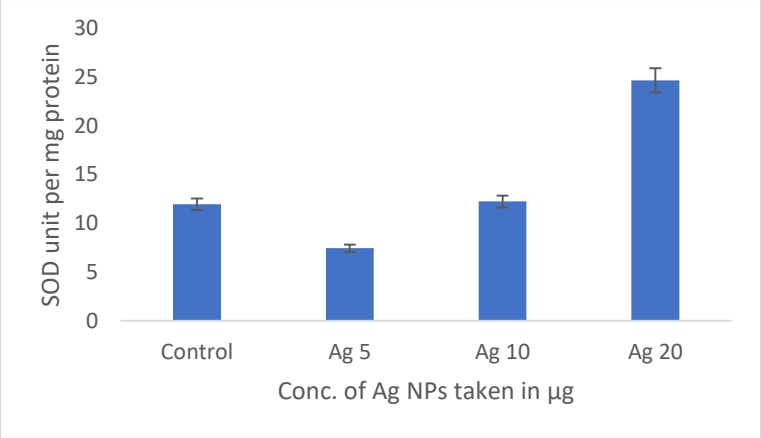

Fig10: Estimation of SOD unit

4.6 Estimation of Peroxidase Activity

Peroxidase activities showed stimulation in response to lower silver concentrations but as its concentration increased. Peroxidase activity was found to be higher in lowest concentration of AgNP.

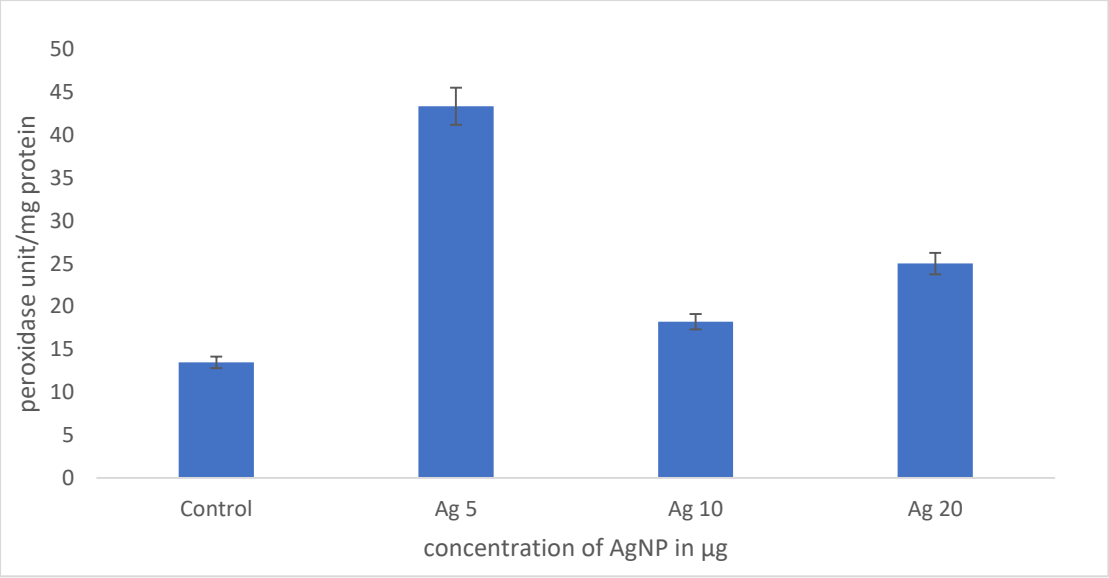

Fig11: Estimation of Peroxidase Activity

4.7 Estimation of Catalase Activity

A native gel was used to estimate the catalase activity. Catalase activity was found to be decreasing with increasing concentration of AgNP.

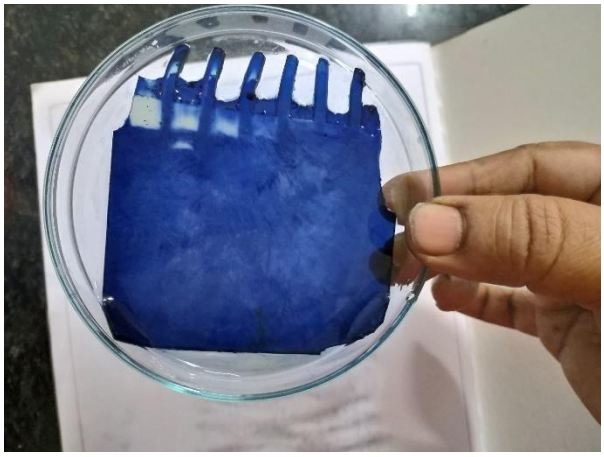

Fig12: Catalase gel activity

4.8 Chlorophyll Estimation

Chlorophyll estimation was done by Arnon's method. There was no significant effect on total chlorophyll content. The OD value were checked for chlorophyll b at 647nm and chlorophyll a at 663nm

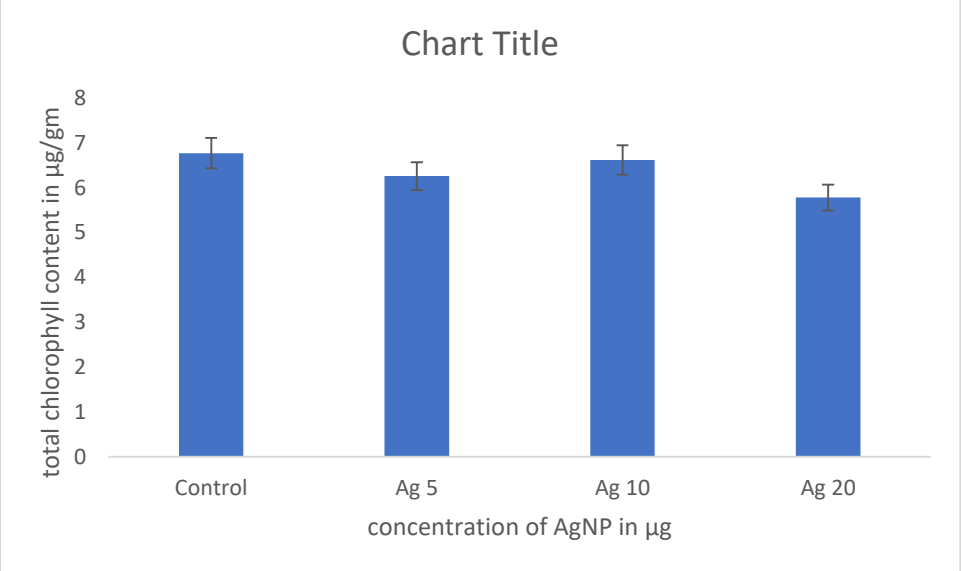

Fig13: Estimation of total chlorophyll content

4.9 Morphological analysis

It was observed that with increase in Ag NPs concentration, the shoot and root lengths also increased by 10%. A significant increase in leaf length was also observed indicating that AgNP

has a stimulating effect on growth of plants which was also observed with increasing protein concentration.

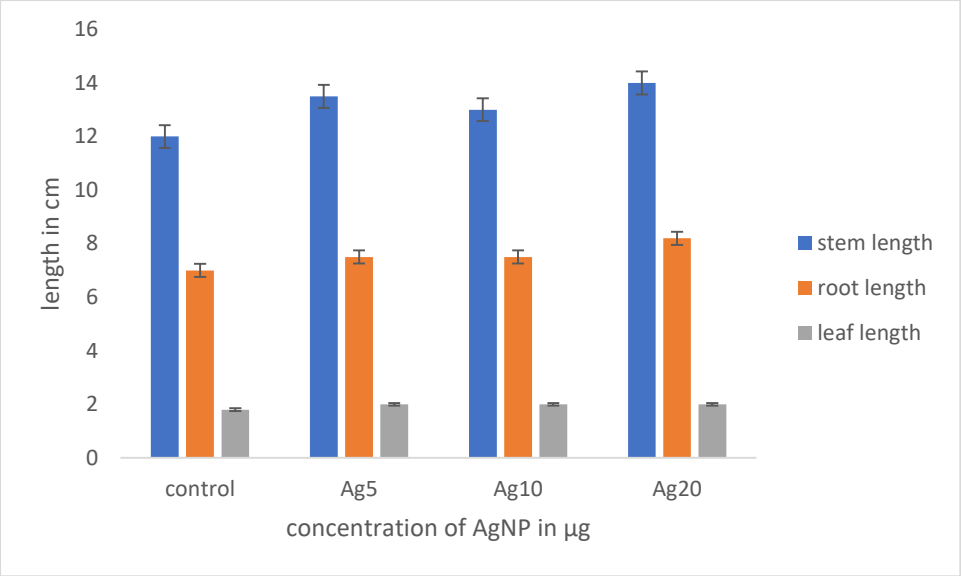

Fig14: Morphological analysis of AgNP treated plants

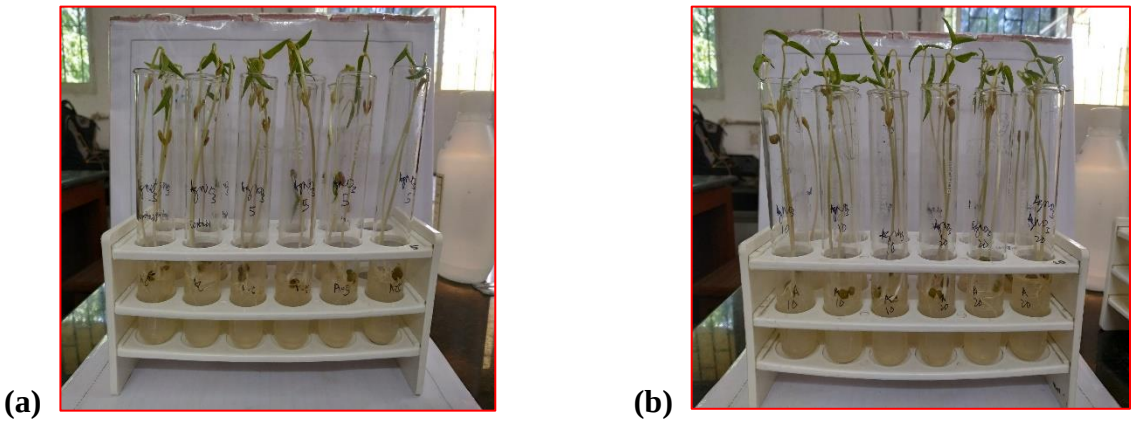

Fig15: Visual observation of treated & non-treated plants- (a)Control & Ag5 (b) Ag10 & Ag20

4.10 ICP-AES Analysis

The concentration of silver nanoparticle was 0.060mg/l and three concentration of silver was taken for the experimental set up to check the absorption of silver by the roots of mung from the agar medium. As it can be seen from the graph of ICP-OES silver nanoparticle were

absorbed maximum at the highest concentration of silver that is 12.97mg/L as compared to 1.355 mg/L in Ag5 and 2.704 mg/L in Ag10.

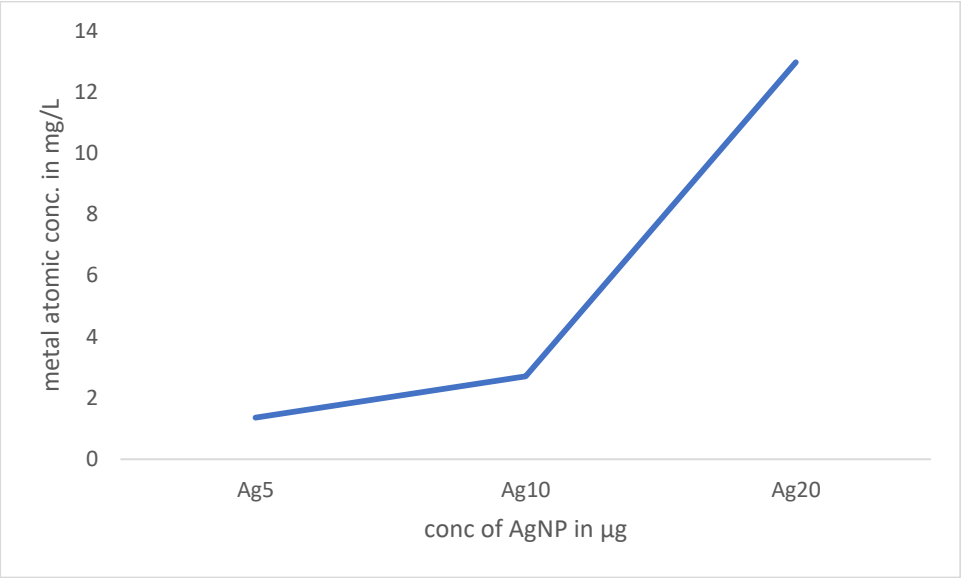

Fig16: ICP-OES analysis of AgNP treated plants

DISCUSSION

Various studies indicate negative, positive or neutral effects of engineered metal nanoparticles on growth and seed germination of higher plants. Obtained results depend on the properties and concentration of nanoparticles and on treated plant specieS. For example, Lin et al. experimentally demonstrated that nanostructured silicon dioxide can be used as a plant growth stimulator of coniferous seedlings. On the other hand, Lin and Xing showed an inhibition of seed germination and root growth by certain NPs in higher plant species. It seems that effects are specific to the type of nanoparticles applied, their concentrations, as well as to the plants tested. Ma et al. argued that the surface area of NPs is a more appropriate estimator of biocidal effects and phytotoxicity than nominal concentration of NPs. Qi et al. [showed that silver-carbon core-shell nanoparticles can enhance Cu uptake in 2-year-old bald cypress (*Taxodium distichum*) seedlings but did not observe significant effects on the growth parameters of the seedlings after 25 weeks of NPs treatment. On the other hand, Lee et al. reported toxicity of silver nanoparticles to two crop species, mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*), as demonstrated by the reduced seedling growth rate. Song et al. (2013) demonstrated that treatment of tomato with Ag NPs resulted in a reduction in biomass and root length. TiO2 NPs could significantly improve the germination rate of seeds; however, bulk TiO2 can have inhibitory effects on seed germination. Similar results were observed in Feizi et al. (2013) and Hawthorne et al. (2012). Because of the high reactivity of small materials, NPs usually show greater toxicity than the same material of larger size (Oberdürster 2000). However, other studies have shown that phytotoxicity increases with particle size. For example, Yasur and Rani (2013) affirmed that all Ag NP treatment groups had no effect on the growth of castor, but in the treatments where the Ag was used in its bulk form, inhibition was observed. This was confirmed by the work of Lee et al. (2010). On the fifth day of NP exposure, synthesized silver NP treatments had higher Ag accumulation than biosynthesized silver NP (B-Ag NP) treatments (10 and 100 mg/L) in water hyacinth; at the high concentration (100 mg/L), B-Ag NPs improved plant growth (Rani et al. 2016).

Similar to the above findings in the current study also the pattern of changes in the activities of antioxidant enzymes (SOD, and peroxidase) differed significantly between experimental variants. In the variant with nanoparticles, SOD activity was 1.5–2.5 higher than in the control at any silver concentration. Peroxidase activities showed stimulation in response to lower silver concentrations but were inhibited as its concentration increased. Higher concentration of protein found in 50ppm AgNP treated plant which

corresponds to peroxidase activity at the same treatment. Protein increase is due to increasing expression of peroxidase activity which may be due to higher level of accumulation of peroxidase enzyme. Regarding generation of oxidative stress during lowest and highest concentration of AgNP ROS generation might be higher which might get eliminated by increasing expression of peroxidase and SOD, during these two treatment respectively. Effect of silver nanoparticle on plant growth promotion in the present investigation can be explained on the basis that mechanisms involved in heavy metal tolerance which may range from exclusion, inclusion and accumulation.

CHAPTER-5

SUMMARY & CONCLUSION

The multitude of bioactive compounds present in the unharnessed agrowaste resource of teak leaves were successfully channeled to synthesize AgNps with a high yield of 97 %. The bioactive compounds were attributed to the reduction of the precursor salt into AgNps and subsequent capping of the AgNps synthesized. Teak leaf aqueous extract mediated AgNps were characterized using UV–vis spectrometry, DLS. The biosynthesized AgNps have potential application in water purifiers, antibacterial fabrics, sports wear and in cosmetics as antibacterial agent and the process used for its synthesis being greener is highly beneficial from environmental, energy consumption and economic perspectives. The presence of biosynthesized AgNPs influences the growth of mung plants at different concentrations. The maximum effect was found at 50 ppm for the AgNP treated plants. Moreover, AgNP as an cofactor is showing an overall positive effect on metabolic activities of the plant to maintain its normal physiological growth which is prevalent in the present study. Hence, we can use silver as a growth promoting agent at certain concentrations for specific plant. However, a complete idea on the mechanism of positive effect can be deduced after underlying studies on a complete signaling system.

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