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GREEN SYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES USING PLANT EXTRACTS AND THEIR ANTINEMIC PROPERTIES

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COIMBATORE – 641 003

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Thesis submitted in part fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY (AGRICULTURE) IN NEMATOLOGY

to the Tamil Nadu Agricultural University, Coimbatore.

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CERTIFICATE

This is to certify that the thesis entitled **"GREEN SYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES USING PLANT EXTRACTS AND THEIR ANTINEMIC PROPERTIES"** submitted in part fulfilment of the requirement for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE) IN NEMATOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research work carried out by **Ms. R. SUREGA**, under my supervision and guidance and that no part of the thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes. However, part of the thesis work has been published in peer reviewed scientific journal of national / international repute (copy enclosed).

Date :
Place: Coimbatore

(Dr. S. RAMAKRISHNAN)
Chairman

Approved by

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	:	**(Dr. S. NAKKEERAN)**

External Examiner:

Date:

ABSTRACT

GREEN SYNTHESIS OF BIOACTIVE SILVER NANOPARTICLES USING PLANT EXTRACTS AND THEIR ANTINEMIC PROPERTIES

By

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Degree : Doctor of Philosophy (Agriculture) in Nematology

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2015

An attempt was made to synthesize the silver nanoparticles (AgNPs) using extracts of chosen three commonly available plants *viz.*, *Tridax procumbens*, *Euphorbia hirta* and *Azardirachta indica* for the green synthesis of bioactive AgNPs. All the three plants accurately yielded AgNPs. Among them, the *T. procumbens* was rated as the most preferred plant source for the green synthesis of AgNPs based on their particles stability and crystalline nature; spherical in shape; smallest size with higher proportion of silver elemental composition and capability of acting as reducing, capping and stabilizing agent of silver nanoparticles.

T. procumbens mediated green synthesized AgNPs influenced attraction, penetration, reduction in egg hatchability and caused significant mortality of juveniles of *Meloidogyne incognita* significantly *in vitro* and its antinemic effect was found to be positively correlated with their concentrations and time of exposure. Similarly, the green synthesized AgNPs using *T. procumbens* had also exhibited antifungal activity against *Fusarium oxysporum* f.sp. *lycopersici* and *Macrophomina phaseolina* and antibacterial against *Ralstonia solanacearum* and *Xanthomonas oryzae* pv *oryzae* and their antimicrobial effect was found to be directly proportional to their concentrations and time of exposure.

In vitro antinemic, antifungal and antibacterial effect exhibited by *T. procumbens* mediated green synthesized AgNPs was confirmed under controlled glasshouse experiments. In these experiments, the plants treated with green synthesized AgNPs significantly checked the root knot nematode *M. incoginta* incidence and fungal disease incidence caused by *F. oxysporum* f.sp. *lycopersici* individually and their disease complex in tomato. In addition, the green synthesized AgNPs also proved to be fungicidal to *M. phaseolina* and effective for the management of nematode fungal disease complex involving *M. incognita* and *M. phaseolina* in blackgram.

Similarly, the green synthesized AgNPs effectively checked the bacterial wilt disease caused by *R. solanacearum* and nematode bacterial disease complex incited by *M. incognita* in association with *R. solanacearum* in brinjal. The significant suppression of nematode/ fungal/ bacterial disease incidence and their disease complex resulted in considerable improvement in plant growth characters and economic yield of tomato, blackgram and brinjal experimented in the present study.

Further, the effect of green synthesized AgNPs was found to be significantly higher than the effect of bio-agent, *Pseudomonas fluorescens* and synthetic chemical pesticides in controlling root-knot nematode, *M. incognita*; fungal pathogens *viz.*, *F. oxysporum* f.sp. *lycopersici*, *M. phaseolina*; bacterial pathogen, *R. solanacearum* and nematode fungal/ bacterial disease complex in tomato, blackgram and brinjal and enhanced their plant biomass under controlled glasshouse conditions.

The profound effect of green synthesized AgNPs using *T. procumbens* in the management of above nematode, fungal and bacterial pathogens and their disease complex with subsequent improvement in plant growth of tomato, blackgram and brinjal was reconfirmed in plant growth chamber.

However, the green synthesized AgNPs using *T. procumbens* were found to be detrimental to all the tested beneficial organisms *viz.,* entomopathogenic nematode, *Steinernema glaseri;* fungal biocontrol agents of *Trichoderma viride* and *Paecilomyces lilacinus* and bacterial biocontrol agents of *Pseudomonas fluorescens* and *Bacillus subtilis.*

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(R.SUREGA)

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CHAPTER I

INTRODUCTION

Agricultural production is continuously reduced worldwide every year due to biotic stress including nematodes. Therefore millions of dollars have been invested in efforts to control the menace caused by the biotic stress. Various natural and artificial methods of control are being practiced for protecting plants from the damage caused by insects, plant disease causing organisms and phytonematodes. Among different methods used for plant pathogens control the use of pesticides is the most prevalent and common practice everywhere. In recent years, environmental hazards caused by excessive use of pesticides have been widely discussed. Therefore scientists in the agricultural field are searching for alternative measures against pesticides (Jo and Kim, 2009 and Sandhya *et al.*, 2014).

Changes in agricultural technology has been a major factor shaping modern agriculture. Consequently, innovative technologies are being introduced in modern agriculture to minimize losses caused by the above biotic stress. Among such technological innovations, nanotechnology is gathering noteworthy considerations due to its robust applications in many fields including agriculture because of its unique physical, chemical and biological properties (Nair *et al*., 2010; Ghormade *et al*., 2011 and Bakshi *et al*., 2014)

The nanotechnology is a broad based science involving manipulation of atoms, electrons, protons and neutrons in a variety of ways to generate new understanding of how materials can be developed to solve many problems in various fields. Several nanodevices/nanomaterial/nanocides have been developed for plant disease prevention and their management (Patolsky *et al*., 2006; Scrinis and Lyons, 2007 and Kawazoe and Meeche, 2005). Hence, the improved properties of the nanoparticles compared to the application of bulk materials have greater opportunity to reduce the load of unwanted chemicals especially plant protectants.

Nowadays many new branches of the nanotechnology are emerging out as nanomedicine, nanobiotechnology, nanorobotics, nanoelectronics etc. The “Nano

Agriculture" will play a major role in the forthcoming years in revolutionizing the field of agriculture (Papaiah *et al.*, 2014). Therefore the significance of nanotechnology has been realized internationally and several countries have set up special committees/groups to support and monitor nanotechnological advancements and harness its benefits for the mankind. The Indian Government had also formulated a Nano Mission during the Eleventh Five Year Plan (Khan and Rizvi, 2014).

In nanotechnology various physical and chemical methods are employed for the synthesis of metal nanoparticles and are being explored and extensively investigated as potential antimicrobials (Shankar *et al*., 2004 and Panacek *et al*., 2006). However, physical and chemical methods used to synthesize metal nanoparticles have certain disadvantages due to involvement of hazardous chemicals, high energy requirements and radiation.

The emerging branch of Bio-Nanotechnology combines biological principles with physical and chemical approaches to produce nano sized particles with specific functions. It also represents an economic substitute for chemical and physical methods of nanoparticles formation (Ahmed *et al*., 2006). Therefore the synthesis of metal nanoparticles using greener methodology and its application in biological fields is a burgeoning field of research (Narayanan and Park, 2014).

The smaller size of the nanoparticles with large surface area enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities (Martinez *et al.,* 2010).

With regard to mechanisms it is speculated that the AgNPs are so small allows them to interact readily with such microorganisms and display multiple modes of inhibitory action against microorganisms (Jung *et al.,* 2010; Lamsal *et al.,* 2011 and Kim *et al.,* 2012). Silver is known to attack a broad range of biological processes in microorganisms including cell membrane structure and functions (Pal *et al.,* 2007). Further, it inhibits the expression of proteins associated with ATP production (Yamanaka *et al.,* 2005). The nanoparticles penetrate the cell, causing damage by interacting with phosphorus and sulfur containing compounds such as DNA and protein (Amarendra and Krishna, 2010). In addition the AgNPs interfere with the enzymatic

metabolism of oxygen which effectively “suffocates” and kills the particular microorganism. Besides the silver suppresses respiration, basal metabolism of electron transfer systems and transport of substrates in the microbial cell membrane (Lamsal *et al*., 2011).

Biological methods of nanoparticles synthesis using microorganisms like algae (Lengke *et al*., 2006), bacteria (Husseiny *et al*., 2007 and Shahverdi *et al*., 2007), fungi (Gajbhiye *et al*., 2009 and Govender *et al*., 2009), actinomycetes (Ahmad *et al*., 2003) and plant or plant extracts (Sumitra, 2013) have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plants for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of maintaining cell cultures (Shankar *et al*., 2004). Further the green synthesis of AgNPs using plant sources which does not require the usage of high pressure, energy, temperature and toxic chemicals is considered as cost effective and ecofriendly method by Barrena *et al*. (2009).

Besides, Parikh *et al*. (2008) and Bar *et al*. (2009) opined that the green synthesis of nanoparticles can be suitably scaled up for large scale with well-defined size and shape. Moreover the ecofriendly nanoparticles were not reported to produce toxic wastes in the process of synthesizing protocol by Mansoori (2005). Many researchers reported the successful synthesis of silver nanoparticles using numerous plant extracts (Krishnaraj *et al*., 2010; Geethalakshmi and Sarada, 2010; Nabikhan *et al*., 2010; Singhal *et al*., 2011; Veerasamy *et al*., 2011; Parasad *et al*., 2011; Logeswari *et al*., 2015; Saxena *et al*., 2012 and Awwad *et al*., 2012).

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction and to act as capping agent. The size of the nanoparticles synthesized using xerophytes, mesophytes and hydrophytes were in the acceptable range of 2- 50 nm (Jha *et al*., 2009).

Hence, Morones *et al*. (2005) concluded that the green synthesis of AgNPs has offered a consistent, nontoxic and ecofriendly approach for plant pathogen management due to their strong antimicrobial properties.

The root knot nematode, *Meloidogyne incognita* (Sasser, 1979 and Taylor and Sasser, 1978), Fusarium wilt causing *Fusarium oxysporum* f. sp. *lycopersici* (Srivastava *et al.,* 2011 and Kirankumar *et al.,* 2008), charcoal rot causing *Macrophomina phaseolina* (Siddiqui and Husain, 1992), bacterial wilt causing *Ralstonia solanacearum* (Hussain and Bora, 2009) and bacterial blight causing *Xanthomonas oryzae* pv *oryzae* (Mew *et al.,* 1993) and their complex disease are widely prevalent in Tamil Nadu and responsible for huge economic loss. Although various management strategies are being suggested to overcome the above problem indepth studies on this line are warranted to evolve better newer techniques.

Hence the present study is programmed with the following objectives

1. To synthesis and characterize silver nanoparticles using plant extracts.
2. To determine the inhibitory property of silver nanoparticles against root- knot nematode.
3. To assess the antifungal and antibacterial activity of green synthesized silver nanoparticles against plant pathogens.
4. To assess the effect of green synthesized silver nanoparticles on beneficial soil microorganisms.

CHAPTER II

REVIEW OF LITERATURE

Nanotechnology is one of the emerging fields of science interfacing life sciences, material science and information technology. The literature pertaining to the new science of Nanotechnology, synthesis and characterization of nanoparticles, their antimicrobial properties against insect pests, fungal and bacterial plant pathogens and phytonematodes and their effect on beneficial microorganisms are reviewed hereunder.

2.1. Genesis of Nanotechnology

Originally the word “Nano” was developed from the Greek word which means ‘dwarf’. Technically the word “Nano” means 10^{-9} or one billionth of metre. Naturally, the word nanotechnology evolved due to use of nanometer size particles (1 to 100 nm). This scale helps to denote that the width of DNA strand is 2.5 nm, size of virus is 100 nm, a red blood cell is 7000 nm and the width of human hair is about 80,000 nm.

The physicist Richard Feynman explained the concept of ‘Nanotechnology’ in the year 1959 and considered as father of Nanotechnology. In continuation, the term "Nanotechnology" was defined by Norio Taniguchi, Professor, Tokyo University of Science in 1974. Accordingly the, nanotechnology mainly consists of the processing of separation, consolidation and deformation of materials by one atom or by one molecule.

2.1.1. Definition for nanotechnology

The National Nanotechnology Initiative has defined nanotechnology as “working at the atomic, molecular and supra molecular levels, in the length scale of approximately 1–100 nm range in order to understand and create materials, devices and systems with fundamentally new properties and functions because of their small structure” (www.nano.gov).

It also defined as an exciting and rapidly emerging technology to manipulate and create tools, materials and structures at the molecular level, often atom by atom into functional structures having nanometre dimensions.

2.1.2. Scope of nanotechnology

Elibol *et al*. (2003) and Salata (2004) stated that the use of nanoparticles (NPs) have greater scope in many fields like electronic, medicine, pharmaceuticals, engineering and agriculture.

2.1.3. General application of nanotechnology in agriculture

The effective use of nanotechnology in agriculture sector is multidirectional and has the potential to change the entire scenario of agriculture with the help of new tools for rapid detection of pathogens and pesticides molecules using nano based kits, treating against pest and disease problems including weeds; improving the ability of plants to absorb nutrients; genetic improvement of plants; conservation of soils and to maintain their structure and fertility of soil (Allah Ditta, 2012).

Hence, Thul *et al.* (2013) concluded that NPs containing herbicides, chemicals or genes which target particular plant parts can be used as 'magic bullets' to enhance the crop productivity.

2.1.4. Specific applications in different branches of agricultural science

The literature scanned on the specific applications of nanotechnology in agricultural science showed promising results of nanotechnology for crop protection as follows.

2.1.4.1. Detection of pest/pathogens

Detection and utilization of biomarkers that accurately indicating the presence of pathogen/s or their reaction is an emerging area of research in nanobiotechnology. In this technology the estimation of differential protein production in diseased plants will be helpful for the detection of pathogens affecting the test plants (Chittaranjan Kole *et al.*, 2013).

2.1.4.2. Nanopesticide formulations

Bergeson (2010) commented that nanopesticides can increase the dispersion and wettability of their formulations and to prevent unwanted pesticide movement. In support of it Bouwmeester *et al.* (2009) and Bordes *et al.* (2009) stated that the nanomaterials and biocomposites exhibited useful properties such as stiffness, permeability, crystallinity,

thermal stability, solubility and biodegradability needed to formulate the nanopesticides. The nanopesticide formulations also offer large specific surface area for increased affinity to the target (Yan *et al.,* 2005). Nanoemulsions, nanoencapsulates, nanocontainers and nanocages were some of the nanopesticide delivery techniques proved to be effective in plant protection programmes (Lyons and Scrinis, 2009).

Nanopesticides can reduce the rate of application because the quantity of product actually being effective is at least 10-15 times smaller than that applied with classical formulations. Hence a much smaller than the normal quantum was sufficient to have much better and prolonged effect for the management of pests of crops.

Clay nanotubes have been developed as carriers of pesticides at low cost for extended release with better contact on plants to reduce the quantum of pesticides by 70-80 per cent in order to reduce the cost of pesticides (Chittaranjan Kole *et al.,* 2013).

2.1.4.3. Pest management

Although nanotechnology have wider application in agriculture very meagre work has been carried out in plant protection. The work carried out so for the management of pest and disease problem indicated the possession of antifungal and antibacterial properties by nanoparticles (Carmen *et al*., 2003). However, almost no information is available about their antinemic properties.

In this context Joseph and Morrison (2006) opined that in the near future nanostructured catalysts play an important role to increase the efficacy of commercially available pesticides and to reduce their level of dosage required to combat pest problem in agricultural and horticultural crops. In continuation the same authors believed that development of nanobiosensors and related smart delivery systems is possible to deliver pesticides as encapsulated nanomaterials for stable and controlled release of biopesticides to fight against different crop pests in the industry of agriculture.

2.2. Synthesis of metal nanoparticles

The conventional methods employed for the synthesis of nanoparticles are illustrated in Fig. 1. The methods employed for the synthesis of nanoparticles are broadly

classified as physical method or top down approach, chemical method or bottom up approach and biological method.

2.2.1. Physical method or Top-down approach

The method involves the breaking down of the bulk material into nanosized structures or particles. The techniques are extension of those that have been used for producing micron sized particles. The top-down approaches are inherently simpler and depend either on removal or division of bulk material or on miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties. The biggest problem with the top-down approach is the imperfection of surface structure as pointed out by Petit *et al*. (1993). For example, nanowires made by lithography were not smooth and contained a lot of impurities and structural defects on its surface. In this method of top down approach involves various instruments *viz.,* high-energy wet ball milling, electron beam lithography, atomic force manipulation, gas-phase condensation, aerosol spray are being used.

2.2.2. Chemical method/Bottom-up approach

Compared to previous physical method the chemical method also known as the bottom up approach was considered as more economical by researchers as this method creates no wastage.

The bottom-up (self-assembly) approach refers to the buildup of a material from the bottom as atom-by-atom, molecule-by-molecule or cluster-by-cluster. Many of these techniques under bottom-up approach are still under development or just beginning to be used for commercial production of nanopowders. The bottom-up approach mainly meant for the chemical synthesis of luminescent nanoparticles involves oraganometallic chemical route, revere-micelle route, sol-gel synthesis, colloidal precipitation, hydrothermal synthesis, template assisted sol-gel, electrodeposition.

2.2.3. Biological method

Many biological systems like bacteria (Kumar and Mamidyala, 2011 and Ganesh Babu and Gunasekaran, 2009), fungi (Rodriques *et al*., 2012) yeast (Seshadri *et al*., 2011)

cyanobacteria (Lengke *et al.,* 2006) actinomycetes (Sastry *et al.,* 2003) and plants (Shankar *et al.,* 2004) have been used for the biological synthesis of nanoparticles (Table 1).

2.2.3.1. Need for green synthesis of nanoparticles

The above conventional methods were expensive and hazardous through liberations of toxic chemicals which are harmful to the environment (Prakash *et al*., 2013 and Rajamanickam *et al*., 2013). The limitations has driven the researchers to develop safe, eco-friendly alternative approaches for the synthesis of NPs. Among the alternative approaches to chemicals, the biological synthesis using plants/plant parts and microorganisms was preferred universally for the synthesis of NPs. Undoubtedly the biological synthesis of NPs have a unique ability for the production of precise shape and controlled structures. The method of green synthesis of NPs not requiring any sophisticated instrumentation was reported as simple, efficient, ecofriendly, inexpensive and safer method by Gurunathan *et al.* (2009). So for many plants were utilized for the synthesis of AgNPs and details of plant/parts used, their concentrations and their effectiveness is tabulated (Table 2).

2.2.3.2. Properties of metal NPs

In general the metallic NPs having a high definite surface area and possess various physicochemical properties of catalytic, optical, electron etc. The metals such as silver (Kim *et al*., 2008 and Kumar *et al*., 2008), copper (Cioffi *et al*., 2005), titanium dioxide (Kwak *et al*., 2001), magnesium, gold (Gu *et al*., 2003), alginate (Ahmad *et al*., 2006) and zinc oxide (Liu *et al*., 2009) are mainly being used for the synthesis of NPs.

2.2.3.4. Silver as best metal nanoparticles

Among the metals being used for the synthesis of NPs the silver is considered as most preferable metal as it possess a distinct antimicrobial, antifungal, antiinflammatory, antiangiogenic and antipermeability properties. Hence, the silver is being commonly used in surgically implanted catheters in order to reduce the infections caused during surgery (Kalishwaralal *et al.,* 2009 and Sheikpranbabu *et al.,* 2009).

Table 1. Biological synthesis of nanoparticles using different sources

S.No	Source	Biomolecules involved	Nanoparticle and size	References
Plants				
1	*Allium cepa*	Vitamin C	Au ~100 nm	Parida *et al*. (2011)
2	*Allium sativum*	Sucrose and fructose	Ag 4 .4 ± 1.5nm	White *et al*. (2011)
3	*Achyranthus aspera*	Polyols	Ag,20-30nm	Daniel *et al*. (2011)
4	*Anacardium occidentale*	Polyols and proteins	Au, Ag, Au-Ag alloy and Au core-Ag shell, 20-60nm	Shenya *et al*. (2011)
5	*Andrographis paniculata*	Hydroxyflavones catechins	Ag, 28nm	Sulochana *et al*. (2012)
6	*Astragalus gummifer*	Proteins	Ag, 13.1±1.0 nm	Kora *et al*. (2012)
7	*Azadirachta indica*	Salanin, Nimbin, Azadirone and Azadirachtins	Au, Ag, 2-100nm	Thirumurugan *et al*. (2010)
8	*Camellia sinensis*	Polyphenolic compounds	Au, 25 nm	Boruah *et al*. (2012)
9	*Carica papaya*	hydroxyflavones and catechins.	Ag, 15nm	Jain *et al*. (2009)
10	*Centella asiatica*	Terpenoid, flavonoid	Ag, 20-45nm	Palaniselvam *et al*. (2012)
11	*Chenopodium album*	Oxalic acid	Ag- 12nm, Au-10 nm	Dwivedi and Gopal (2011)
12	*Coleus aromaticus*	Flavonoids	Ag- 40–50 nm.	Vanaja and Annadurai (2012)
13	*Cinnamomum zeylanicum*	Terpenoids	Pd,15 to 20 nm	Sathishkumar *et al*. (2009)
14	*Cinnamomum camphora*	Polyols, heterocyclic components	Pd, 3.2to 6.0 nm	Xin *et al*. (2010)
15	*Citrullus colocynthis*	Polyphenols with aromatic ring and bound amide region	Ag, 31nm	Satyavani *et al*. (2011)
16	*Datura metel*	Plastohydroquinone or plastrocohydroquinol	Ag, 16 to 40 nm	Kesharwani *et al*. (2009)
17	*Desmodium triflorum*	Water-soluble antioxidative agents like ascorbic acids	Ag, 5–20 nm	Ahamed *et al*. (2011)
18	*Dioscorea bulbifera*	Polyphenols or flavonoids	Ag, 8–20 nm	Ghosh *et al*. (2012)

S.No	Source	Biomolecules involved	Nanoparticle and size	References
19	*Dioscorea oppositifolia*	Polyphenols with aromatic ring and bound amide region	Ag, 14nm	Maheswari *et al*. (2012)
20	*Elettaria cardamomom*	Alcohols, carboxylic, acids, ethers, esters and aliphatic amines	Ag,31 nm	Gnanajobitha *et al*. (2012)
21	*Gardenia jasminoides*	Geniposide, chlorogenicacid,crocins and crocetin	P3-5nm	Jha *et al*. (2009)
22	*Glycyrrhiza Glabra*	Flavonoids, terpenoids	Ag-20 nm	Dinesh *et al*. (2012)
23	*Hibiscus cannabinus*	Ascorbic acid	Ag-9 nm	Bindhu and Umadevi (2013)
24	*Hydrilla verticilata*	Proteins	Ag 65.55nm	Sable *et al*. (2012)
25	*Jatropha curcas*	Curcacycline (anonapeptide) Curcain	ZnS -10nm Pb 10-12.5 nm	Hudlikar *et al*. (2012) Joglekar *et al*. (2011)
26	*Justicia gendarussa*	Polyphenol and lavonoid	Au-27 nm	Fazaludeena *et al*. (2012)
27	*Lantana camara*	Carbohydrates, glycosides and flavonoids	Ag-12.55	Sivakumar *et al*. (2012)
28	*Leonuri herba*	Polyphenols and hydroxyl groups	Ag 9.9 to 13.0nm	Rang *et al*. (2012)
29	*Mentha piperita*	Menthol	Ag - 90nm Au- 150nm	Ali *et al*. (2011)
30	*Mirabilis jalapa*	Polyols	Au-100nm	Vankar and Bajpai (2010)
31	*Morinda pubescens*	Hydroxyflavones, catechins	Ag 25-50nm	Mary and Inbathamizh (2012)
32	*Ocimum sanctum*	Phenolic and flavanoid compounds, Proteins Ascorbic acid, gallic acid, terpenoids	Ag~10 nm Ag, 4–30nm Pt- 23 nm	Ahmad *et al.* (2010) Ramteke *et al.* (2013) Soundarrajan *et al*. (2012)
33	*Parthenium hysterophorus*	Hydroxyflavones and catecns	Ag-10nm	Ashok Kumar (2012)
34	*Pedilanthus tithymaloides*	Proteins and enzymes	Ag15-30nm	Sundaravadivelan and Nalini (2012)
35	*Piper betle*	Proteins	Ag-37 nm	Mallikarjuna *et al*. (2012)
36	*Piper nigrum*	Proteins	Ag-5- 50 nm	Garg (2012)
37	*Plumeria rubra*	Proteins	Ag-32 nm	Patil *et al*. (2012)

S.No	Source	Biomolecules involved	Nanoparticle and size	References
38	*Sesuvium portulacastrum*	Proteins, flavones and terpenids	Ag- 5-20nm	Nabikhan *et al*. (2010)
39	*Solanum xanthocarpum*	Phenolics, alkaloids and sugars	Ag-10 nm	Amin *et al*. (2012)
40	*Glycine max*	Proteins and amino acids	Pd~15 nm	Petla *et al*. (2012)
41	*Swietenia mahogany*	Polyhydroxy limonoids	Ag, Au	Mondal *et al*.(2011)
42	*Syzygium aromaticum*	Flavonoids	Au-5-100nm	Deshpande *et al*. (2010)
43	*Terminalia catappa*	Hydrolysable tannins	Au10-35nm	Ankamwar (2010)
44	*Trianthema decandra*	Hydroxyflavones and catechins.	Ag10-50nm	Geethalakshmi and Sarada (2010)
45	*Tridax procumbens*	Water-soluble carbohydrates	Cuo_2	Gopalakrishnan *et al*. (2012)
46	*Vitus vinifera*	Flavone and anthocyanins	Pb- 61nm	Pavani *et al*. (2012)
47	*Zingiber officinale*	alkanoids, flavonoids	Ag-10nm	Singh *et al*. (2011)

ii). Microorganisms

Bacteria			
1.	*Bacillus cereus*	Ag 5nm	Ganesh Babu and Gunasekaran (2009)
2.	*Bacillus thuringiensis*	Ag 10/20nm	Jain *et al*. (2010)
3.	*Escherichia coli*	Ag 30/50nm	Gurunathan *et al.* (2009)
4.	*Escherichia coli*	Cds	Sweeney *et al*. (2004)
5.	*Lactobacillus strains*	Ag,Au 15/40nm	Sintubin *et al*. (2009)
6.	*Pseudomonas stutzeri*	Ag>200nm	Klaus *et al*. (1999)
7.	*Corynebacterium*	Ag 5/15nm	Zhang *et al*. (2005)
8.	*Staphylococcus aureus*	Ag 150/180nm	Nanda and Saravanan (2009)
9.	*Ureibacillus thermosphaericus*	Ag 1/100nm	Juibari *et al*.(2011)
Fungi			
1.	*Aspergillus niger*	Ag 20nm	Gade *et al*. (2008)
2.	*Aspergillus oryzae*	Ag 5-50nm	Binupriya *et al*. (2010)
3.	*Fusarium oxysporum*	Ag 1/5nm	Duran *et al*. (2007)
4.	*Fusarium solani*	Ag 5/35nm	Ingle *et al*.(2009)
5.	*Pleurotus sajorcaju*	Ag 5/50nm	Nithya and Ragunathan (2009)

6.	*Trichoderma viride*	Ag 10/40nm	Thakkar *et al*. (2010)
7.	*Klebsiella pneumoniae*	Se 100/400nm	Fesharaki *et al*.(2010)
Yeast			
1.	Silver-tolerant strain MKY3	Ag 2/20nm	Kowshik *et al*. (2003)
2.	*Candida glabrata*	CdS 50/150nm	Dameron *et al*. (1989)
3.	*Schizosaccharomyce pombe*	CdS 50/150nm	Dameron *et al*. (1989)
4.	*Extremophillic yeast*	Ag	Mourato *et al*. (2011)
5.	*Rhodospiridium dibovatum*	PbS	Seshadri *et al*. (2011)
iii) Others			
1.	DNA	Au/CdS	Mahtab *et al*. (1995); Shaiu *et al*. (1993)
2.	Proteins	Au	Safer *et al*. (1986); Hainfeld and Furuya (1992)
3.	Immunoglobulins, serum albumins	Au	Shenton *et al*. (1999); Beesley (1989)

Table 2. Details on green synthesized silver nanoparticles and their antimicrobial properties

S.No.	Name of the plants	$AgNO_3$ concentration mode and period of plant extraction	Part used	Activity	References
1	*Acalypha indica*	1 mM Boiling 5 min	Leaf	antifungal	Krishnaraj *et al*. (2012)
2	*Albizia adianthifolia*	1 mM Boiling 15 min	Leaf	anticancer	Gengan *et al.* (2013)
3	*Allium sativum*	1 mM Boiling 5 min	Clove	-	Ahamed *et al*. (2011)
4	*Anacardium occidentale*	0.1mM RT 5 min	Leaf	-	Shenya *et al*. (2011)
5	*Annona squamosa*	1mM Rotary shaker 1h	Peel	-	Kumar *et al*. (2012)
6	*Areca catechu*	1 mM 180 sec in microwave	Dried Nuts	-	Bhat *et al*. (2013)
7	*Artemisia nilagirica*	1-6 mM Boiling 5 min	Leaf	antibacterial	Vijayakumar *et al.* (2013)
8	*Artocarpus heterophyllus*	6 mM Boiling 60 min	Seed	antibacterial	Jagtap and Bapat (2013)
9	*Azadirachta indica*	10 mM 30 -240 min	Leaf	-	Prathna *et al*. (2011)
10	*Boswellia serrata*	1 mM	Gum	antibacterial	Kora *et al*. (2012)
11	*Catharanthus roseus*	1 mM Boiling 10 min	Leaf	-	Kannan *et al*. (2011)

S.No.	Name of the plants	$AgNO_3$ concentration mode and period of plant extraction	Part used	Activity	References
12	*Catharanthus roseus*	1 mM Boiling 5 min	Leaf	antiplasmodial	Ponarulselvam *et al*. (2012)
13	*Callicarpa maingayi*	10 mM 80% methanol 72 h	Stem bark	-	Shameli *et al*. (2012)
14	*Cissus quadrangularis*	1mM 60^{o}C 5 min	Stem	antiparasitic	Santhosh kumar *et al*. (2012)
15	*Citrus sinensis*	1 mM Boiling 2 min	Peel	antibacterial	Kaviya *et al*. (2011b)
16	*Cleome viscosa*	3 mM Boiling 2 min	Leaf	-	Lakshmi (2011)
17	*Cocos nucifera*	1 mM Room temp	Coir	-	Roopan *et al*. (2013)
18	*Cynodon dactylon*	1 mM Boiling 2-3 min	Leaf	antibacterial	Sahu *et al*. (2012)
19	*Eclipta prostrata*	1mM Boiling 5 min	Leaf	antimalarial	Rajakumar and Rahuman (2011)
20	*Euphorbia nivulia*	10mM Boiling 30 s (microwave)	Stem latex	antibacterial	Valodkar *et al*. (2011)
21	*Hevea brasiliensis*	-	Latex	-	Guidelli *et al*. (2011)
22	*Hibiscus cannabinus*	5 mM Boiling 5 min	Leaf	antibacterial	Bindhu and Umadevi, (2013)
23	*Iresine herbstii*	1 mM Boiling 5 min	Leaf	antibacterial antioxidant	Dipankar and Murugan, (2012)
24	*Lawsonia inermis*	1 mM	Leaf	antilousicidal	Marimuthu *et al*. (2012)
25	*Macrotyloma uniflorum*	0.1m M Room temp 2 min	Seed	-	Vidhu *et al*. (2011)
26	*Malva parviflora*	1 mM 70% ethanol 7 days	Leaf	-	Zaved *et al*. (2012)
2	*Mangifera indica*	0.1 mM Boiling 1 min	Leaf	-	Philip (2011)
28	*Manilkara zapota*	1 mM Boiling 5 min	Leaf	Pest control	Kamaraj *et al*. (2012)
29	*Medicago sativa*	1 mM Soaking 10 mM	Seed	antibacterial	Lukman *et al*. (2011)
30	*Melia azedarach*	1 mM 30-95^{o}C 10 Min	Leaf	anticancer	Sukirtha *et al*. (2012)
31	*Menth piperita*	1 mM Boiling 10 Min	Leaf	antibacterial	Mubarak *et al*. (2011)
32	*Morinda pubescens*	1 mM Boiling 15 Min	Leaf	antioxidant anticancer	Inbathamizh *et al*. (2013)

S.No.	Name of the plants	$AgNO_3$ concentration mode and period of plant extraction	Part used	Activity	References
33	*Morinda citrifolia*	1 mM Boiling 15 Min	Root	cytotoxicity	Suman *et al*. (2013)
34	*Mukia scabrella*	1 mM 65ºC 20 min	Leaf	antibacterial	Prabakar *et al*. (2013)
35	*Murraya koenigii*	1 mM Boiling 3 min	Leaf	-	Christensen *et al*. (2011)
36	*Ocimum sanctum*	1 mM Boiling 5 min	Leaf	-	Subba Rao *et al*. (2013)
37	*O. tenuiflorum*	1mM Boiling	Leaf	antimicrobial	Logeswari *et al*. (2015)
38	*Panicum virgatum*	1 mM Boiling 3 min	Grass	-	Mason *et al*. (2012)
39	*Piper betle L.*	1 mM Boiling 5 min	Leaf	-	Usha Rani and Rajasekharreddy (2011)
40	*Pithecellobium dulce*	10 mM 60 ºC 5 min	Leaf	larvicidal	Raman *et al*. (2012)
41	*Prosopis juliflora*	10 mM Boiling 15 min	Leaf	antimicrobial	Raja *et al*. (2012)
42	*Punica granatum*	10mM Dried 60 ºC	Peel	-	Jebakumar and Sethuraman (2013)
43	*Rhizophora apiculata*	1 mM 30-95 oC	Dried Leaf	antibacterial	Antonya *et al*. (2011)
44	*Solanum torvum*	Sohlet extraction	Fruit	antibacterial antioxidant	Ramamurthy *et al*. (2013)
45	*Solanum trilobatum*	1 mM Boiling 3 min	Leaf	antidandruff activity	Pant *et al*. (2013)
46	*Terminalia chebula*	10 mM 50ºC, 2 min	Fruit	methylene blue reduction	Jebakumar and Sethuraman (2013)
47	*Tribulus terrestris*	1 mM Boiling 10 min	Fruit	antimicrobial	Gopinath *et al*. (2012)
48	*Vitex negundo L.*	2 mM methanol 2 h	Leaf	anticancer HCT15	Prabhu *et al*. (2013)
49	*Withania somnifera*	1 mM 30 min on sand bath	Leaf	-	Nagati *et al*. (2012)

2.2.3.5. Advantages of green synthesized AgNPs using plants

The major advantage of using plant extracts for the synthesis of AgNPs is that they are easily available, safe and nontoxic in most cases, have a broad variety of metabolites that can aid in the reduction of silver ions and are quicker than microbes during the synthesis. The plants assisting reduction of ions due to their phytochemicals was considered as major mechanism of green synthesis of AgNPs. The main

phytochemicals responsible for the immediate reduction of the ions includes water soluble phytochemicals of terpenoids, flavones, ketones, aldehydes, amides, carboxylic acids, organic acids, quinones. Therefore, Jha *et al.* (2009) suggested that the phytochemicals were involved directly in the reduction of the ions and formation of AgNPs.

2.2.3.6. Mechanisms involved in green synthesis of AgNPs

In the environmentally safer technique of green synthesis of AgNPs using plant sources, the plants were acting as reducing, capping and stabilizing agents of AgNPs. In this process the reduction of Ag^+ ions was made by the combination of biomolecules *viz.*, enzymes/proteins/amino acids/polysaccharides/vitamins present in plant/parts. According to Duran *et al*. (2005) the enzyme nitrate reductase in the nitrogen cycle was responsible for the conversion of nitrate to nitrite. Anil Kumar *et al*. established the first direct evidence for the involvement of nitrate reductase in the synthesis of AgNPs in the year 2007. Subsequently Kalimuthu *et al*. (2008) confirmed that the enzyme "Nitrate reductase" present in plant system was mainly responsible for the synthesis of AgNPs.

In the green synthesis of AgNPs the use of specific enzyme NADPH dependent nitrate reductase play an important role to synthesize AgNPs homogeneously during downstream processing. During this process the nitrate was converted into nitrite as an electron was shuttled to the incoming silver ions and it was described excellently with the bacterium, *Bacillus licheniformis*. The bacterium was known to secrete the cofactor NADH and NADH dependent enzymes especially nitrate reductase. The enzyme was reported to be responsible for the bioreduction of Ag^+ to Ag^0 and the subsequent formation of AgNPs by Kalimuthu *et al*. (2008).

2.2.3.7. Favorable conditions for the green synthesis of AgNPs

The green synthesis of AgNPs was proved to be faster towards alkaline conditions compared to acidic conditions. However, the synthesis reported to reaches its maximum at pH 10 and started to decline thereafter. Further at alkaline conditions there is no need for agitating the mixture for the formation of AgNPs and all the silver ions converted to AgNPs within 30 min.

The proteins involved in the synthesis bind with silver at thiol regions (–SH) forming a –S–Ag bond indicating the conversion of Ag^{+} to Ag^{0}. In addition, the alkaline ion (OH) was reported to be essential for the reduction of metal ions. Generally it took four days for the production of silver ions under neutral pH whereas it was very much less than an hour when the pH was alkaline (Sanghi and Verma, 2009).

2.2.4. Characterization of AgNPs

Generally the synthesized nanoparticles were characterized using Particle size analyzer with Zeta potential, Ultraviolet–Visible Spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) with Energy Dispersive X-Ray Spectroscopy (EDAX), Fourier Transform Infrared Spectroscopy (FTIR) and X- ray Diffraction (XRD).

The above sophisticated instruments working with different principles were helpful to know the synthesized nanoparticles size, shape, stability, elemental nature and functional groups etc. as follows (Table 3).

2.2.4.1. Particle size analyzer with Zeta potential

2.2.4.1.1. Measurement of particles size

The particle size analyzer is used to determine the size of particles and their distribution pattern of synthesized nanoparticles.

The instrument works on the principle of Dynamic Light Scattering. In this principle when a beam of light (a laser) is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size i.e. when particle size is smaller the angle of light scattering will be larger.

2.2.4.1.2. Stability of particles

The particle size analyzer with Zeta potential is essential to determine the stability of synthesized NPs. The stability of the nanoparticles was increasing with increase in the zeta potential and a minimum of ± 30 mV zeta potential was required to indicate the stable nature of particles (Jacobs and Muller, 2002 and Jebakumar and Sethuraman, 2012).

2.2.4.2. Ultraviolet – Visible (UV-Vis) Spectroscopy

The UV-Vis spectroscopy is a valuable tool used for confirming the synthesis of AgNPs which are stable in nature.

It is well known that the optical absorption spectra of metal nanoparticles are dominated by surface plasmon resonances (SPRs) that shift to longer wavelengths with increasing particle size. Also, it is well known that the absorbance of AgNPs mainly depends on size and shape of particles. In general, the number of SPR peaks decreased with increase in the symmetry of the nanoparticles. The position and shape of the plasmon absorption depends on the particles size and shape and the dielectric constant of the surrounding medium (Kelly *et al*., 2003 and Rai *et al*., 2006). The appearance of SPR peaks at 430-460 nm confirmed the formation of AgNPs as spectroscopic signature (Jenkins and Snyder, 1996; Basavaraja *et al*., 2008).

2.2.4.3. Scanning Electron Microscopy (SEM)

The SEM analysis is employed to characterize the size, shape, morphology and elemental composition of synthesized AgNPs by focusing a beam of high-energy electrons to generate a variety of signals at the surface of solid specimen of AgNPs. The signals generated from electron-sample interactions revealed information about the external morphology (texture), chemical composition and orientation of materials making up the sample of AgNPs in the form of images. (Ashok Kumar, 2012 and Ramamurthy *et al*., 2013).

2.2.4.4. Transmission Electron Microscopy (TEM)

The TEM is being used to estimate the synthesized nanoparticles size, shape and their distribution (Zaved *et al*., 2012).

The TEM is a microscopic technique wherein a beam of electron is transmitted through an ultra-thin specimen making it to interact with the specimen. Then an image formed over imaging screen by the electrons transmitted through the specimen, magnified and focused by an objective lens.

2.2.4.4.1. SEM/TEM with Energy Dispersive X-Ray Spectroscopy (EDAX)

The EDAX is an analytical technique used for the analysis of elemental composition and chemical nature of nanoparticles (Zaved *et al*., 2012).

The interactions between electromagnetic radiation and AgNPs emitting analytical rays in response to being hit with charged particles is the principle of EDAX. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an elements atomic structure to be identified uniquely from one another.

2.2.4.5. X- Ray diffraction (XRD)

XRD is a technique to determine the phase composition of a sample, crystal structure, texture or orientation. The principle of XRD is that the X-rays are passed through a material and the pattern produced give information of size and shape of the unit cell. The atoms are crystal in structure arranged in a periodic array and thus can diffracted light at different angle. When X-ray passing through a crystal it produces a diffraction pattern that diffract ion gives the information about the atomic arrangement within the crystals. In silver nanoparticles, the XRD gives nature and purity of the particles (Bindhu and Umadevi, 2013).

2.2.4.6. Fourier Transform Infrared (FTIR) Spectroscopy

The instruments FTIR is used for the identification of probable biomolecules responsible for acting as reducing, capping and stabilizing agents of AgNPs in the given samples (Vidhu *et al*., 2011).

Its principle is used to determine the reduction process with biomolecules while green synthesizing nanoparticles using aqueous plant extract and also bonding interaction between themselves. Besides it is also helpful to detect the vibration characteristics of chemical functional groups of the green synthesized AgNPs by adsorbing infrared radiation in a specific wave number range.

2.2.5. Samples characterized for the synthesis of AgNPs

Around two dozen of economic and uneconomic plants comprising of herb, shrub and trees were used for the green synthesis of AgNPs during the last five years. The details of synthesized nanoparticles characterized for their size and shape of the AgNPs is given in Table. 3.

Table 3. Characterization of green synthesized AgNPs

S. No	Plant used	SEM	TEM	References
1.	*Ananas comosus*	--	Spherical and oval shape NPs with size ranging from 10 to 15 nm.	Ahmad and Sharma (2012)
2.	*Azardirachta indica*	--	Spherical shape with average size of 43-59 nm	Gavhane *et al.*, 2012
3.	*Catharanthus roseus*	Spherical shape NPs with size ranging from 35 to 55 nm.	--	Ponarulselvam *et al.* (2012)
4.	*Chrysanthemum morifolium*	--	Spherical shape NPs with an average size of 20 nm–50 nm	He *et al*. (2013)
5.	*Cocos nucifera* L.	Spherical shape	Spherical shape NPs with average size of 23 ± 2 nm	Roopan *et al.* (2013)
6.	*Datura alba* Nees.	High density silver NPs in spherical shape	--	Abasaheb *et al*. (2013)
7.	*Desmodium triflorum*	--	Particle size in the range of 5–20 nm, spherical shape.	Ahmad *et al*. (2011)
8.	*Euphorbia hirta*	Spherical shape NPs with the diameter ranging from 40-50 nm.	--	Elumalai *et al*. (2010)
9.	*Ipomoea carnea* Jacq.	Aggregates of Spherical silver NPs	--	Abasaheb *et al*. (2013)
10.	*Lantana camara*	Particle size of 39.60 nm	--	Thirumurugan *et al*. (2010)
11.	*Morinda citrifolia L.*	Spherical shape NPs the size ranges from 10 to 60 nm in diameter	10–60 nm with the average size of 29 nm	Sathishkumar *et al*. (2012)
12.	*Ocimum tenuiflorum*	--	Irregular shape with size ranging from 10-50 nm.	Kiruba Daniel *et al*. (2011)
13.	*Odina wodier* Roxb.	Uniform spherical shape NPs with the size ranging from 5-30 nm	Size - 5 and 40 nm	Arun kumar *et al*. (2013)
14.	*Olea europaea*	Cubical shape of NPs with diameter range 10-30 nm	Quasi spherical NPs with an average size of 30 ±6 nm	Awwad *et al*. (2012) and Khalil *et al*. (2013)

S. No	Plant used	SEM	TEM	References
15.	*Panicum virgatum*	Spherical, rod-like, triangular, pentagonal, and hexagonal shape Ag NPs with the size ranging between 20 and 40 nm.	--	Mason *et al.* (2012)
16.	*Parthenium hysterophorus*	--	Irregular shape with the size of 30 to 80 nm	Parashar *et al.* (2009)
17.	*Polyalthia longifolia*	--	Size - 50 nm and 35 nm	Kaviya *et al.* (2011a)
18.	*Tridax procumbens*	Size – 55 nm	--	Dhanalakshmi and Rajendran (2012)
19.	*Withania somnifera*	High density Ag NPs	The particle size ranging between 5-40 nm spherical shape	Nagati *et al.* (2012)

2.3. Role of nanoparticles in plant protection

2.3.1. Plant pathogens

Considerable work has been carried out on the effect of nanoparticles against disease causing plant pathogens like fungi and bacteria compared to insects and phytonematodes. The information available in the literature on the effects of NPs is summarized below.

2.3.1.1. Fungal plant pathogens

In an experiment ZnO and MgO NPs were evaluated against *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Mucor plumbeus* at different concentrations. The study revealed that higher concentrations of NPs were most effective than lower concentrations to inhibit the germination of spores of above fungi (Wani and Shah, 2012).

Similarly Wani and Shah (2012) also reported that higher concentrations of NPs with magnesium, iron and zinc *in vitro* inhibited spores germination of *Penicillium notatum*, *Aspergillus niger* and *Nigrospora oryzae*. The same trend was also confirmed by Yehia and Ahmed (2013) with ZnO NPs against two pathogenic fungi *viz.*, *Fusarium oxysporum* and *P. expansum.*

2.3.1.2. Post harvest pathogens

He *et al*. (2011) reported that ZnO NPs @ 3 mmol/L significantly inhibited the growth of two postharvest pathogenic fungi *viz., Botrytis cinerea* and *P. expansum* compared to *B. cinerea*. In this study the pathogen *P. expansum* was found to be more sensitive to the treatment with ZnO NPs. Further the authors reported that the ZnO NPs inhibited the growth of *B. cinerea* by causing deformation of fungal hyphae and by affecting their cellular functions. In addition the ZnO NPs were also found to prevent the development of conidiophores and conidia of *P. expansum* which eventually led to the death of fungal hyphae.

Chowdappa *et al*. (2014) synthesized AgNPs using chitosan as reducing and stabilizing agent and evaluated against *Colletotrichum gloeosporioides* in mango (cv. Alphonso). In this study the authors proved that postharvest decay in mango can be minimized by chitosan mediated AgNPs and concluded that the technology has commercial value.

2.3.2. Antimicrobial properties of NPs with special reference to AgNPs

2.3.2.1. Antinemic

2.3.2.1.1 On free living nematode

The free living nematode is the most frequently studied nematode for the influence of AgNPs (Yeon Roh *et al.*, 2009; Lim *et al.*, 2012; Meyer *et al.*, 2010 and Yang *et al*., 2012). The results of the study indicated that the AgNPs affecting behavior and development of the nematodes was toxic to *C. elegans*.

2.3.2.1.2 On plant parasitic nematodes

2.3.2.1.2.1 *Meloidogyne incognita*

In an experiment conducted *in vitro* and under controlled pot conditions for the effect of AgNPs, SiO_2 and TiO_2 against *M. incognita.* It is observed that there was cent per cent immobility and mortality of J_2 of root knot nematode by the above NPs with 800, 400 and 200 mg ml/lit except SiO_2. However under pot experimentations AgNPs and TiO_2 were found to reduce the shoot height and root length of tomato compared to untreated control. Hence the authors opined that NPs are causing toxic effect on tomato (Ardakani, 2013).

Cromwell *et al*. (2014) observed that more than 99 per cent of J_2 of *M. incognita* became inactive on exposure to AgNPs in water at 30 to 150µg/ml under laboratory conditions.

2.3.2.1.2.2. *M. graminis*

In turfgrass AgNPs reduced *M. graminis* population in soil by 92 and 82 per cent after 4 and 2 days of exposure under field conditions. Similarly the biweekly application of 90.4 mg/m^2 of AgNPs caused reduction in gall formation in root and improved quality of turfgrass for two years without phytotoxicity (Cromwell *et al*., 2014).

2.3.2.1.3 On beneficial nematodes

2.3.2.1.3.1. Entomopathogenic nematodes (EPN)

2.3.2.1.3.1.1 *Steinernema* sp.

The AgNPs with the concentrations of 0.5 and 5 ppm were reported to be toxic to *Steinernema feltiae* (IJs) under laboratory conditions and the mortality of EPN was found to concentration dependent. However in the case of *Alphitobius diaperinus* the EPN which survived on contact with nanoparticles were able to infect insects as reported by Kucharska *et al*. (2009).

The copper nanoparticles also found to cause mortality of *S. feltiae.* In this study the per cent mortality of nematodes was increasing with increase in their concentrations and period of exposure to NPs. Further the NPs pathogenic properties over *A. diaperinus* was confirmed by Kucharska *et al.* (2014). Besides the authors reported that the high concentrations of Cu decreased abilities of EPNs to enter, develop and proliferate inside the host body.

2.3.2.1.3.1.2. *Heterorhabditis* sp.

The AgNPs caused mortality of IJs of *Heterorhabditis bacteriophora* and its lethal effect of AgNPs was dependent on the concentrations and period of exposure (Kucharska *et al.*, 2009).

2.3.2.2. Antifungal

2.3.2.2.1. *In vitro*

2.3.2.2.1.1. AgNPs

Gajbhiye *et al.* (2009) evaluated the effect of AgNPs along with fluconazole for the antifungal activity against *Phoma glomerata, P. herbarum, F. semitectum*, *Trichoderma* sp. and *Candida albicans*. In this study the authors observed that the antifungal activity of fluconazole enhanced against the test fungi in the presence of AgNPs. The fluconazole in combination with AgNPs showed the maximum inhibitory effect against *C. albicans* followed by *P. glomerata* and *Trichoderma* sp. whereas no significant enhancement in the inhibitory effect was noticed against *P. herbarum* and *F. semitectum*.

Various forms of silver ions and nanoparticles were tested for their antifungal activity against plant pathogenic fungi *viz., Bipolaris sorokiniana* and *Magnaporthe grisea*. The study conducted by Jo and Kim (2009) indicated that both forms inhibited colony formation of above pathogens. In this assay the effective concentrations (EC50) of the silver compounds to inhibit the colony formation was found to be higher for *B. sorokiniana* than for *M. grisea*.

The fungal hyphal growth of sclerotium forming species *viz., Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *S. minor* inhibited remarkably by AgNPs in a dose dependent manner. The antimicrobial property of the AgNPs was differing among the fungi and it was in the order of *R. solani* > *S. sclerotiorum* > *S. minor* (Min *et al*., 2009). A study on fungal sclerotial germination revealed that the nanoparticles had significant inhibitory effect. Further it is stated that the sclerotial germination of *S. sclerotiorum* was most effectively inhibited at lower concentrations of AgNPs. In addition it is observed that the hyphae of *S. sclerotiorum* damaged severely by the AgNPs and resulting in the separation of layers of hyphal wall and collapsing of hyphae. Hence the authors suggested the possibility of using AgNPs as an alternative to pesticides for the management of scleotium forming phytopathogenic fungi.

Kim *et al*. (2012) treated silver colloidal solution at the concentrations of 10, 25, 50, and 100 ppm against eighteen different plant pathogenic fungi on potato dextrose agar, malt extract agar and corn meal agar plates. The results indicated that AgNPs possess antifungal properties against these plant pathogens at 100 ppm.

Green synthesized AgNPs using *Acalypha indica* leaf extracts were tested on fungal plant pathogens *viz., Alternaria alternata*, *S. sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia lunata*. The results showed that 15 mg concentration of AgNPs showed excellent inhibitory activity against all the tested pathogens (Krishnaraj *et al*., 2012).

Sahayaraj *et al*. (2012) reported that plant based AgNPs inhibited the growth of *Fusarium oxysporum* f.sp. *vasinfectum* affecting cotton. Elumalai and Vinothkumar (2013) demonstrated the effectiveness of biogenic synthesized AgNPs using aqueous extract of shade dried leaves of *Conyza ambigua* against *Aspergillus niger, A. flavus* and *S. rolfsii*.

Banadkouki *et al*. (2013) found that AgNPs was effective for the management of *F. solani* causing dry rot of potato under storage conditions and also suggested the opt time of application of AgNPs for the management of the fungus. Bholay *et al*. (2013) documented that there is a synergistic effect when fluconazole combined with AgNPs in the management of *A. alternate*, *F. oxysporum* and *Cladosporium herbarum.*

Pulit *et al*. (2013) proved that nanosilver at 50 ppm inhibited the growth of *C. cladosporoides* and *A. niger* by 90 and 70 per cent respectively. Narayanan and Park (2014) evaluated the green synthesized NPs against wood degrading fungal pathogens *viz., Gloeophyllum abietinum*, *G. trabeum*, *Chaetomium globosum* and *Phanerochaete sordida* and proved its effectiveness.

Papaiah *et al*. (2014) considered that metallic AgNPs as an alternative to synthetic chemicals for the management of stem rot causing *S. rolfsii*, dry root rot causing *R. bataticola* and collar rot causing *A. niger.*

Ouda (2014) reported that AgNPs and CuNPs were effective against *A. alternata* and *B. cinerea*. In this study it is observed that 15 mg/lit exhibited maximum inhibitory effect on the growth of fungal hyphae through damaging its hyphae and conidia.

2.3.2.2.1.2. Combined effect of AgNPs with other metal NPs

Almost no work has been carried out on the combined effect of AgNPs with other metal NPs against plant pathogenic fungi. However Ouda (2014) observed higher effectiveness of AgNPs against *A. alternata* and *B. cinerea* when compared to AgNPs in combination of CuNPs.

The AgNPs at 100 ppm caused maximum inhibitory effect on the growth of fungal hyphae as well as conidial germination of anthracnose *Colletotrichum* sp. in pepper and fungal pathogens responsible for causing powdery mildew on cucumber and pumpkin (Lamsal *et al*., 2011).

2.3.2.2.2. Under greenhouse conditions

Nanosilver at 7 ppm exhibited inhibitory effect on *S. cepivorum* by more than 90 per cent and increased biomass and dry weight of onion (Jung *et al.*, 2010).

The spraying of AgNPs on rice seedlings was highly effective against *M. grisea* according to Elamawi *et al*. (2013). In this study the SEM image revealed the AgNPs caused detrimental effect on the growth of fungal mycelia.

Sandhya *et al*. (2014) noticed complete inhibition of conidial germination of *B. sorokiniana* causing spot blotch disease of wheat by AgNPs. In this experiment the histochemical studies revealed deposition of lignin in vascular bundles due to AgNPs.

The nanosized silica silver consisting of nanosilver and silica molecules at 3 ppm exhibited antifungal activity against many phytopathogenic fungi *viz., B. cinerea, R. solani, C. gloeosporioides, M. grisea* and *Pythium ultimum*. In contrast, a number of beneficial bacteria or plant pathogenic bacteria *viz., Escherichia coli, Bacillus subtilis, Pseudomonas syringae* pv. *syringae, Xanthomonas campestris* pv. *vesicatoria, Azotobacter chrococuum and Rhizobium tropici* were found to be affected at higher concentration of 100 ppm of nanosized silica silver (Park *et al*., 2006). However the authors concluded that the effectiveness of nanosized silica silver was higher in respect of pathogen causing powdery mildews of pumpkin.

2.3.2.2.3. In plant growth chamber

Both ionic and AgNPs significantly checked the fungal pathogens *viz.*, *B. sorokiniana* and *M. grisea* on perennial ryegrass. In plant growth chamber both silver ions and AgNPs influenced spore colonization and progression of disease. The study concluded that the efficacy of silver ions and AgNPs was greater. Therefore, it can be used as preventive measure since it promotes the direct contact with spores and germ tubes and affect their viability (Jo and Kim, 2009).

2.3.2.2.4. *In vivo*

It is observed that AgNPs inhibited *Colletotrichum* sp. in a field experiment conducted by Lamsal *et al*. (2011). On observations through SEM it is understood that AgNPs causing detrimental effect on mycelial growth of the fungus.

Park *et al.* (2006) evaluated nanosized silica silver (0.3 ppm) against pathogen causing powdery mildew of pumpkin and found to be effective under field conditions.

2.3.2.3. Antibacterial properties

2.3.2.3.1. *In vitro*

The green synthesized AgNPs with *Ulva fasciata* inhibited the growth of *X. campestris* pv. *malvacearum* with zone of inhibition of 14.00±0.58 mm. In this study the minimum inhibitory concentration was fixed as 40.00±5.77µg/mL (Rajesh *et al*., 2012). Similarly another plant based AgNPs was reported to inhibit the growth of same bacterium with 10.33±0.33 mm zone of inhibition by Sahayaraj *et al*. (2012).

The green synthesized AgNPs using leaf and fruit extracts of oak (*Quercus infectoria*) showed antibacterial activity against the plant pathogenic bacteria *viz.*, *Pectobacterium carotovorum*, *Ralstonia solanacearum*, *Erwinia amylovora* and *X. citri* (Mahmood Chahardooli *et al*., 2014).

2.3.2.3.2. Under glasshouse conditions

The DNA-directed AgNPs developed by Ismail *et al*. (2013) effectively decreased the disease incidence of bacterial spot caused by *X. perforans* in tomato. In this study

even low concentration of 16 ppm of AgNPs had significant advantages of improved stability and stronger adsorption properties with excellent antibacterial effect.

The general antimicrobial properties of AgNPs is furnished in Table 4.

2.3.3. Mechanism of action of AgNPs

2.3.3.1. Antinemic

The information available on the mechanism of AgNPs in the suppression of nematode population is very meagre. The available information also implying the effect of nanoparticles at gene level. Therefore indepth study needs to be documented on the mechanism of nanoparticles in the management of nematodes.

2.3.3.1.1. Suppression in nematode reproduction

Yeon Roh *et al.* (2009) investigated the ecotoxicity of AgNPs on *Caenorhabditis elegans*. In this study the authors reported that AgNPs at 0.1 and 0.5 mg/lit exerted considerable toxicity on *C. elegans* as the nanoparticles decreased reproductive potential of the nematode and increased expression of the superoxide dismutases-3 *(sod-3)* and abnormal dauer formation protein (*daf-12*) genes. Functional genomic studies using mutant analyses also proved the expressions of *sod-3* and *daf-12* gene and it has been related to failure in the reproductive potential of *C. elegans*. The oxidative stress was also attributed as an important mechanism for the toxicity of AgNPs.

Lim *et al.* (2012) elaborately studied the mechanism of AgNPs on *C. elegans* by focusing the involvement of oxidative stress for the failure of reproductive capacity of nematodes. Initially the AgNPs tested as potential oxidative stress inducers increased the formation of reactive oxygen species (ROS) in *C. elegans*. Subsequently the potential upstream activated signaling pathway was studied in response to AgNPs exposure by paying special attention to the *C. elegans* PMK-1 p38 mitogen activated protein kinase (MAPK). The results indicated that AgNPs exposure led to increased ROS formation, increased expression of PMK-1 p38 MAPK and hypoxia inducible factor (HIF-1), glutathione S-transferase (GST) enzyme activity decreased reproductive potential in *C. elegans*. Therefore, the results suggested that oxidative stress is an important mechanism of AgNPs induced reproduction toxicity on *C. elegans* and that PMK-1 p38

MAPK played an important role in this mechanism. Similarly Yang *et al*. (2012) reported that AgNPs which were less soluble due to size or coating with polyvinylpyrrolidone and gum arabic also acted via oxidative stress to affect target organism of *C. elegans*.

2.3.3.1.2. Suppression in nematode development

Meyer *et al.* (2010) studied the physicochemical, behavior, uptake, toxicity and mechanism of AgNPs with *C. elegans* being used as biological model and concluded that AgNPs inhibited the growth and development of the nematode.

2.3.3.2. Antifungal

2.3.3.2.1. Effect on cells

The silver ions known to produce ROS were causing damage to proteins, lipids and nucleic acids which were detrimental to fungal cells (Hwang *et al.,* 2008).

Min *et al.* (2009) reported that the AgNPs seriously damage the fungal hyphal walls which resulted in the plasmolysis of hyphae. Such damage to the surface of the fungal hyphae leads to release of internal cellular materials and resulting in the shrinkage of hyphae.

2.3.3.2.2. Effect on DNA

It is hypothesized that Ag^{+} primarily affects the function of membrane bound enzymes such as those in the respiratory chain (Kim *et al.*, 2012).

The silver ions made the DNA to lose their ability to replicate. Such reaction resulting in inactivated expression of ribosomal subunit proteins as well as certain other cellular proteins and enzymes essential for ATP production (Hwang *et al.,* 2008).

2.3.3.2.3. Effect on metabolism and physiology

The AgNPs disrupt the transport systems including ion efflux in fungi according to Morones *et al.* (2005). The disruption of ion efflux by AgNPs causes rapid accumulation of silver ions thereby interrupting cellular processes such as metabolism and respiration of fungi by reacting at molecular level.

2.3.3.2.4. Effect on fungal reproduction

Min *et al.* (2009) observed that fungal conidial germination was inhibited by AgNPs in general.

2.3.3.3. Antibacterial

The antibacterial activity exhibited by AgNPs depends on $AgNO_3$ concentration. The nanoparticles having larger surface area readily interact with microorganism and the same was explained as probable reason for their bactericidal effect by Baker *et al*. (2005).

2.3.3.3.1. Effect on cells

The cell membrane of microorganisms like bacteria is negatively charged and AgNPs are positively charged. Hence, when positively charged AgNPs accumulated on negatively charged bacterial cell membrane it results in substantial conformational change in the membrane. Therefore, the bacterial cells ultimately losses their permeability control which leads to death of the same (Ramamurthy *et al.,* 2013 and Hamouda and Baker 2000).

Amro *et al.* (2000) reported that AgNPs affecting the bacterial cell outer membrane and thereby change the permeability of cell membrane. Due to this interactional effect there was progressive release of lipopolysaccharides and membrane proteins from bacterial cells which leads to death of cells.

Danilczuk *et al.* (2006) and Kim *et al.* (2007) observed formation of free radicals induced membrane damage in bacteria and attributed the same as mechanism of AgNPs exhibiting antimicrobial property.

Sondi and Sondi (2004) and Morones *et al.* (2005) commented that the nanoparticles themselves release silver ions in the bacterial cells to act as bactericide. Mubarak Ali *et al.* (2011) particularly stated that once AgNPs entered the bacterial cell, they started to interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrates which was critical for cell viability and cell division. In continuation, the AgNPs was reported to attack the respiratory chain, cell division and cause death of bacterial cells by Mahendra *et al.* (2009).

2.3.3.3.2. Effect on DNA

In addition the DNA became inactivated due to loss in their replication ability, changes in the expression of ribosomal subunits proteins as well as some other cellular proteins and enzymes essential for ATP production (Sanghi and Verma, 2009).

However, Guzman *et al.* (2012) considered interaction of AgNPs with biological macromolecules such as enzymes and DNA through an electro release mechanism as possible reason for the antibacterial activity of AgNPs. The authors proved that the nanoparticles attached to the cell membrane penetrated inside the bacteria having sulfur containing proteins and interacted with the proteins of the cell as well as phosphorus containing compounds like DNA. Such type of interaction damaged DNA and proteins caused death of cells. In this process the Ag^{+} bound to functional groups of proteins also results in protein denaturation.

Table 4. Antimicrobial properties of AgNPs

S.No	Microorganisms	Size of AgNPs	Doses	Effect	References
1	*Acinetobacter baumanii*	20-25 nm	0.4 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
2	*Aspergillus niger*	20-25 nm	25 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
3	*Azotobacter chrococuum*	20nm	100µg/ml	Inhibitory	Park *et al*. (2006)
4	*Bacillus cereus*	4.5 nm	200 µg/ml	Resistant	Ghazvini *et al*. (2008)
5	*Bacillus subtilis*	20nm	100µg/ml	Inhibitory	Park *et al*. (2006)
6	*Bipolaris sorokiniana*	20-30nm	500 µg/ml	Inhibitory	Ki Jo and Kim (2009)
7	*Botrytis cinerea*	20nm	10µg/ml	Inhibitory	Park *et al*. (2006)
8	*Candida albicans*	20-25 nm	6.4 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
9	*Candida glabrata*	3nm	1-7 µg/ml	LC80	Jun *et al*. (2008)
10	*Candida krusei*	3nm	13 µg/ml	LC80	Jun *et al*. (2008)
11	*Candida neoformans*	20-25 nm	3 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
12	*Candida parapsilosis*	3nm	4-25 µg/ml	LC80	Jun *et al*. (2008)
13	*Candida tropicalis*	3nm	7 µg/ml	LC80	Jun *et al*. (2008)
14	*Colletotrichum gloeosporioides*	20nm	10µg/ml	Inhibitory	Park *et al*. (2006)
15	*Escherichia coli*	100nm	60 µg/ml	Inhibitory	Raffi *et al* (2008)

S.No	Microorganisms	Size of AgNPs	Doses	Effect	References
16	*Magnaporthe grisea*	20-30nm	200 µg/ml	Inhibitory	Ki Jo and Kim (2009)
17	*Magnaporthe grisea*	20nm	10µg/ml	Inhibitory	Park *et al*. (2006)
18	*Mycobacterium smegmatis*	20-25 nm	0.5 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
19	*Mycobacterium bovis*	20-25 nm	1.1 µg/ml	Inhibitory	Gutierrez *et al*. (2010)
20	*Pseudomonas aeruginosa*	18-34nm	7.95 µg/ml	Inhibitory	Shavandi *et al*. (2010)
21	*Pseudomonas syringae*	20nm	100µg/ml	Inhibitory	Park *et al*. (2006)
22	*Pythium ultimum*	20nm	10µg/ml	Inhibitory	Park *et al*. (2006)
23	*Raffaelea* sp	4-8nm	25 µg/ml	Inhibitory	Woo *et al*. (2009).
24	*Rhizobium tropici*	20nm	100µg/ml	Inhibitory	Park *et al*. (2006)
25	*Rhyzoctonia solani*	20nm	10µg/ml	Inhibitory	Park *et al*. (2006)
26	*Rhyzoctonia solani*	4-8nm	7µg/ml	Inhibitory	Min *et al*. (2009)
27	*Staphylococcus aureus*	20-30nm	33 µg/ml	Inhibitory	Kim *et al*. (2007)
28	*Streptococcus mutans*	8.4 nm	101.9µg/ml	Inhibitory	Cristobal *et al*. (2009)
29	*Trichoderma harzianum*	18-34nm	150 µg/ml	Inhibitory	Gavanji *et al*. (2012)
30	*Trichoderma viride*	18-34nm	150 µg/ml	Inhibitory	Gavanji *et al*. (2012)
31	*Trichophyton mentagrophytes*	3nm	2-4 µg/ml	LC80	Jun *et al*.(2008)
32	*Xanthomonas compestris*	20nm	100µg/ml	Inhibitory	Park *et al*. (2006)

2.4. Effect of AgNPs on beneficial soil microbes

No considerable work has been carried out on the illeffects of AgNPs over beneficial soil microbes being helpful for the management of pest and disease as biocontrol agents to fix atmospheric nitrogen etc. Therefore, it is not desirable to draw a conclusion on the illeffect of AgNPs with the limited work carried work on this line as detailed below.

2.4.1. Fungi

The different concentrations of AgNPs inhibited the growth rate of beneficial fungi *viz., T. viride* and *T. harzianum* and their effect positively related with the time of exposure and concentrations of AgNPs (Gavanji *et al*., 2012).

2.4.2. Bacteria

Park *et al*. (2006) found that the AgNPs caused cent per cent growth inhibition on beneficial bacteria like *B. subtilis* and *Azotobacter chroococum*. Subsequently Srivathsan *et al*. (2012) studied the general effect of AgNPs on soil inhabiting bacteria and reported that the AgNPs inhibited the colonization of rhizosphere bacteria.

CHAPTER III

MATERIALS AND METHODS

The materials and methods used for the present study on green synthesis of silver nanoparticles, their characterization; antinemic effect on *Meloidogyne incognita*; antifungal effect on *Fusarium oxysporum* f.sp. *lycopersici* and *Macrophomina phaseolina* and antibacterial effect on *Ralstonia solanacearum* and *Xanthomonas oryzae* pv *oryzae in vitro*; management of above nematode, fungal and bacterial pathogens and their disease complexes under glasshouse conditions and in plant growth chamber; effect on beneficial nematodes, fungus and bacteria are furnished below.

3.1. Selection of plants

The commonly available plants *viz., Tridax procumbens* L., *Euphorbia hirta* L., and *Azadirachta indica* A.Juss were selected for green synthesis of silver nanoparticles in the present study.

3.1.1. Preparation of plant extracts

The healthy fresh leaves of above *T. procumbens, E. hirta* and *A. indica* were collected from farm area of the Tamil Nadu Agricultural University, Coimbatore. The leaves were thoroughly washed with tap water to remove soil, dirt, etc. and finally with distilled water. Twenty five grams of the leaves were cut into small pieces and taken in 250 ml Erlenmeyer flask containing sterile distilled water and kept in a water bath at 90°C for 45 min. The extract was filtered using Whatman filter paper no.1 to obtain the aqueous plant extracts.

3.1.2. Preparation of silver nitrate solution

The silver nitrate ($AgNO_3$) was purchased from Hi-Media Laboratories Pvt. Ltd. was used in the present study. A concentration of 0.01M $AgNO_3$ solution was prepared by dissolving 0.169g $AgNO_3$ in 100 ml double distilled water and used for the green synthesis of silver nanoparticles (AgNPs).

3.1.3. Synthesis of AgNPs

The above filtered aqueous leaf extracts of 25 ml was added individually to 100 ml of 0.01M of $AgNO_3$ in a 250 ml Erlenmeyer flask and boiled at 90^oC for 10-15 min. Then subsequently content of the flask was stirred at 150 rpm at 30^oC using magnetic stirrer. The process was continued till the change of colour occurred from yellowish to dark brown indicating the completion process of AgNPs synthesis. Then the extract was centrifuged at 6000 rpm for 15 min and the resultant pellet was kept in hot air oven overnight at 60^oC to make a fine powder of nanoparticles (Dhanalakshmi and Rajendran, 2012).

The various steps involved in the green synthesis of AgNPs are illustrated in Plate 1.

3.1.4. Characterization of the green synthesised AgNPs

The characterization of green synthesized AgNPs was performed using different instruments as shown in Plate 2.

3.1.4.1. Particle size analyser with Zeta potential

3.1.4.1.1. Particle size

The particle size analyser considered as a very fast, reliable and reproducible technique to measure a very wide range of nanoparticles.

In the present study the particle size analyser (Make: HORIBA Nanoparticle analyser SZ-100 Japan) was used to measure the size and distribution pattern of green synthesized AgNPs.

A sample of 0.5 mg of AgNPs was dispersed in 20 ml distilled water and sonicated for 32 min (4 cycles) using ultrasonicator make of Citizon Digital Cleaner at 42,000 Hz frequency and 5 ml of the dispersed AgNPs taken in the cuvette was loaded in the particle size analyser to determine the size of green synthesised AgNPs.

3.1.4.1.2. Stability of particles

The stability of the AgNPs was assessed in terms of Zeta potential using the above particle size analyser allowing to run from -200 mV to +200 mV and plotting the data in graph.

3.1.4.2. UV-Vis Spectroscopy

The UV-Vis spectroscopy was used to confirm the synthesis of AgNPs based on their optical properties.

The wavelength of green synthesised AgNPs based on light absorbance in the range of 300 to 600 nm with resolution of 1nm was measured by loading the sample of 2 ml in quartz cuvette in UV-Vis spectroscopy (Make: SPECORD 210 PLUS, Germany).

3.1.4.3. Scanning Electron Microscope (SEM)

In the present study the SEM (Make: FEI QUANTA 250, Netherlands) was used to characterize the size and morphology of the nanoparticles.

SEM has the magnification ranging from 20X to approximately 30,000X with spatial resolution of 50 to 100 nm and for the imaging a sample of 0.5 mg AgNPs was dusted on one side of the double sided adhesive carbon conducting tape. The tape was then mounted on 8 mm dia aluminium stub. The sample was observed at different magnification and the images were picturized.

3.1.4.4. Transmission Electron Microscope (TEM)

The TEM (Make: FEI TECHNAI SPRIT, Netherlands) was used to image the green synthesised AgNPs.

For the above purpose of picturizing image, a sample of 0.5 mg of AgNPs was dispersed in 20 ml distilled water and sonicated for 32 min by using Citizon Digital Ultrasonic Cleaner at 42,000 Hz frequency. A drop of the resultant solution was placed on 300-mesh lacy carbon coated copper grid, dried and the images were captured at different magnifications.

3.1.4.4.1. Energy Dispersive X-Ray Spectroscopy (EDAX)

The EDAX of SEM and TEM was used for elemental analysis or chemical characterization of green synthesised AgNPs. The quantitative analysis of AgNPs samples was done by FEI QUANTA 250 EDAX and FEI TECHNAI SPRIT. About 0.5 mg of AgNPs sample was dusted on the carbon conducting tape. Then the tap was mounted on sample stage and the images were taken in SEM and TEM.

3.1.4.5. X-ray Diffraction (XRD)

The XRD (Make: Bruker D8 Advance Powder X-ray Diffractometer, Germany) analysis is helpful to know phase structure and purity of the green synthesized AgNPs. It is generally used as common technique for studying the phase composition of AgNPs, its crystalline/amorphous structure and texture or orientation.

For XRD analysis, one g of AgNPs was loaded in the sample holder of instrument and allowed to run at 40 kV voltage and 30 mA current with Cu K (a) radiation for the identification of nature of the particles (Shankar *et al.*, 2004). Then the diffractogram was recorded with 2θ value ranging between 10-80^{o} at a scanning speed of 0.080 for a step time of a second at room temperature (25^{o}C).

The result obtained from the XRD pattern was interpreted with standard reference of Joint Committee on Powder Diffraction Standards (JCPDS card number 04-0783) for the characterization of AgNPs.

3.1.4.6. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR (Make: Shimadzu, Japan) is a chemical analytical method meant for the measurement of infrared intensity, wavelength or wave number of light of the green synthesised AgNPs.

Therefore for FTIR analysis a sample of 0.5 g of AgNPs was used and the spectra was scanned in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} for the characterization of chemical functional groups present over the surface of green synthesised AgNPs.

The FTIR data measures the interaction between Ag and protein molecules. Therefore, when an infrared light interacts with the sample of AgNPs, the chemical bonds show stretching, contracting and bending nature of nanoparticles as indicated by peak in FTIR and the result was matched with the standard library search of Infrared charts.

3.1.4.7. Comparison of plant based AgNPs based on their characterization

The characters *viz.*, particle size, shape, stability, structure, elemental composition etc. of green synthesised AgNPs using *T. procumbens*, *E. hirta* and *A. indica* were

compared. Among them the most effective plant based AgNPs was selected based on desirable characters and advanced for their antimicrobial properties.

3.2. Antimicrobial properties of green synthesised AgNPs

3.2.1. Against root knot nematode *Meloidogyne incognita*

3.2.1.1. Maintenance of pure culture *M. incognita*

The inoculum required for raising the pure culture of *M. incognita* was obtained from tomato plants raised under glasshouse conditions (28±2^{o}C). The roots of tomato with conspicuous galls were selected, washed gently and thoroughly in water and examined for the presence of egg masses under microscope. Galls which showed the protruding bodies of the matured females covered with gelatinous matrix were dissected and a single egg mass was transferred to the embryo cups half filled with water. The juveniles hatched out from the egg mass were collected and inoculated on four weeks old tomato (Var: PKM1) seedlings for maintaining the pure culture of root knot nematode. The pots were maintained under glasshouse conditions and watered regularly.

The plants were uprooted 45 days after inoculation and washed free of soil. The well-developed egg masses were carefully teased out and transferred to Petri dish containing adequate amount of distilled water and incubated under laboratory conditions. The eggs and juveniles that hatched out were used for different experiments conducted in the present study.

3.2.1.2. On egg hatching

Different concentrations of the green synthesised AgNPs using *T. procumbens* were sonicated for 45 min in ultra sonicator to disperse the nanoparticles uniformly in double distilled water. Five ml of each concentrations of AgNPs were transferred to sterilized Petri dishes (5 cm dia) containing uniform sized egg mass of *M. incognita* @ one/Petri dish to evaluate the antinemic effect of AgNPs on inhibition of egg hatching of *M. incognita.* All the treatments were replicated four times in Completely Randomized Design (CRD) and compared with water as untreated control. Observations were made at 24 hr interval for 7 days to calculate the per cent inhibition in egg hatching of *M. incognita.*

3.2.1.3. On juveniles

As above the different concentrations of AgNPs were taken separately in sterilized Petri dish (5 cm dia) @ 5ml/Petri dish. The freshly hatched second stage juveniles (J_2) of *M. incognita* obtained from pure culturing of root knot nematode were transferred @ 100 J_2/ Petri dish to study the effect of AgNPs on the juveniles of *M. incognita* under laboratory conditions (28±2^{o}C). Each treatment replicated four times in CRD to assess the larvicidal effect of AgNPs.

Observations were recorded on mortality of juveniles of *M. incognita* at 24 hr interval for a period of week and the per cent mortality of juveniles was worked out after confirming their irreversible reaction of *M. incognita* on exposure to the green synthesised AgNPs.

3.2.1.4. On attraction of *M. incognita*

The attraction/repulsion effect of different concentrations of AgNPs on *M. incognita* in tomato was studied *in vitro* using agar plate method (Azmi and Jairajpuri, 1976).

Three circles with 3, 2 and 1cm radius were drawn from the centre of Petri dish on the bottom and denoted as regions of a, b and c respectively to indicate the per cent attraction of *M. incognita* juveniles as 25, 50 and 75 respectively towards the root bits of tomato placed at the centre of Petri dish. Then melted water agar (2%) was poured on the Petri dishes and kept in an incubator at constant temperature of 28^{o}C for 24 hr. Thereafter the surface sterilized roots of tomato (Var. PKM1) using mercuric chloride at 0.1% were dipped with different concentrations of AgNPs, cut into small bits of 1cm length and placed at the centre of each Petri dish. The root bits without nanoparticles was served as untreated control.

The freshly hatched juveniles (J_2) of *M. incognita* were inoculated in each Petri dish near the periphery @ 100 J_2 / Petri dish maintained at room temperature (28 ± 2^{o}C).

Observations on the number of juveniles of *M. incognita* attracted was counted region wise at 24, 48, 72, 96 and 120 hr after introduction.

3.2.1.5. On root penetration of *M. incognita*

An experiment was conducted in a plant growth chamber maintained at 25± 2ºC, 12,000 lux for nine hours and 75 per cent relative humidity to assess the influence of AgNPs on root penetration of *M. incognita* in the nanoparticluated roots of tomato. The sterilized glass tubes of 7.5 cm was filled with steam sterilized fine river sand treated with different concentrations of AgNPs. The soil without AgNPs filled in test tube was served as untreated control. Tomato seeds (Var: PKM1) surface sterilized with the mercuric chloride at 0.1 per cent were sown @ four seeds per test tube. The experiment was conducted in CRD with suitable replications.

All the glass tubes were inoculated with 100 J_2 of *M. incognita/* tube after two weeks of sowing and thinned to one seedling per tube. The tomato plants in the tubes were maintained for a week. The plants from each tube were removed carefully at an interval of 24 hr starting from the day after inoculation (DAI) and continued upto six days. The roots were cut into small bits of 1cm length, immersed in boiled lactophenol acid fuchsin, destained with plain lactophenol and examined under microscope for studying the root penetration ability of *M. incognita* (McBeth *et al*., 1941).

3.2.2. Antifungal activities of green synthesised AgNPs *in vitro*

3.2.2.1. Against *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina*

The antifungal activity of green synthesised AgNPs *in vitro* was evaluated against *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina*. The pure culture of both fungal pathogens were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, (TNAU) Coimbatore, sub cultured and maintained on potato dextrose agar (PDA) medium. The antifungal assay was conducted using poisoned food technique using PDA medium amended with different concentrations of green synthesised AgNPs using *T. procumbens*. The PDA medium without the amending AgNPs was served as untreated control. A disc of 9 mm of actively growing mycelial mat of 7 days old culture of *F. oxysporum* f.sp. *lycopersici* and *M. phasolina* was placed separately at the centre in each of the AgNPs amended medium as well as in the untreated control and incubated at 28 ± 2˚C for five days. Each treatment was replicated four times in CRD to assess the antifungal property.

The mycelial growth of the pathogens was measured for five days after inoculation. The per cent inhibition in the mycelial growth over untreated control was calculated to express the antifungal activity as follows.

3.2.2.1.1. Assessment of per cent inhibition (PI)

$$PI = \frac{C-T}{C} \times 100$$

The radial growth of fungal mycelium was measured in mm. The per cent inhibition of the test fungal pathogens of *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* was calculated as follows.

Where, 'C' is the growth of test pathogen (mm) in the absence of the AgNPs; 'T' is the growth of test pathogen (mm) in the presence of the AgNPs.

3.2.3. Antibacterial activities of the green synthesised AgNPs *in vitro*

3.2.3.1. Against *R. solanacearum* and *X. oryzae* pv *oryzae*

The antibacterial activities of green synthesised AgNPs using *T. procumbens* were evaluated against *R. solanacearum* and *X. oryzae* pv *oryzae*. The culture of test bacterial pathogens were obtained from the Department of Agricultural Microbiology and Plant Pathology, TNAU, Coimbatore.

The antibacterial assay was conducted by agar well diffusion method using Nutrient Agar (NA) medium amended with different concentrations of AgNPs. The cell suspension of *R. solanacearum* and *X. oryzae* pv. *oryzae* were prepared using sterile distilled water to a concentration of 10^8 cfu/ml. One ml of the above bacterial cell suspension was mixed separately with 19 ml of NA medium and poured on to the sterile Petri dishes. After solidification, sterile paper disc (9 mm dia) were placed on the surface of the medium 1 cm away from the side of the Petri dish as treatment and at centre as control followed by the application of 20 µl of the sonicated green synthesised AgNPs per disc. Each treatment was replicated four times in CRD (Winstead and Kelman, 1952). The plates were incubated at 28 ± 2^{o}C and the zone of inhibition of bacterial cell growth was measured with scale 24 hr after inoculation and expressed in mm.

3.2.4. Ultra structural changes induced by the green synthesised AgNPs

3.2.4.1. On nematode

The SEM has a several fold increase in resolution over the light microscope and an exceptional depth of field is currently being used to describe the surface features of microscopic organisms like nematodes. Therefore a study was undertaken with SEM to understand the ultra-structural changes caused by the green synthesised AgNPs on root knot nematode *M. incognita.*

Usually the biological wet sample like nematodes does not require any prior sample processing. Hence, the AgNPs treated and untreated teased out egg mass and juvenile stages of *M. incognita* taken for the present study were placed directly on the carbon coated stub and introduced in to the chamber of SEM. Observations on structural integrity of egg mass and juvenile of nematodes were made under low vaccum condition by focusing the samples through SEM at various resolutions ranging from 2500-6000X.

3.2.4.2. On fungus

A carbon coated stub with double-side adhesive carbon tape gently touched over the mycelial mat grown in the Petri dishes of the experiment meant for studying the antifungal effect of the green synthesised AgNPs on *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* as mentioned in the section 3.2.3 was taken as sample.

The test fungal pathogen samples were introduced into the chamber of SEM for observing the ultra-structural changes at various resolutions of 2500-6000X.

3.3. Influence of green synthesised AgNPs in the management of plant pathogens

3.3.1. Under glasshouse conditions

3.3.1.1. Management of *M. incognita* on tomato

Experiments were conducted under glasshouse conditions to study the influence of green synthesised AgNPs for the management of *M. incognita* in tomato as follows.

Four weeks old healthy tomato seedlings were transplanted (Var: PKM1) at 2 seedlings/pot filled with 5 kg stream sterilized pot mixture prepared with red soil, sand and farm yard manure in 2:1:1 proportion. One week after transplanting the seedlings

were thinned to one per pot followed by imposing treatments and inoculating with freshly hatched J_2 of *M. incognita* @ one J_2/g soil by making three slanting holes to a depth of 2-3 cm around the seedlings. All the following treatments were replicated five times in CRD.

Details of treatments

T_1 : Silver nanoparticles at 0.0005%

T_2 : *Pseudomonas fluorescens* @ 2 .5kg/ha

T_3 : Carbofuran 3G @ 1Kg a.i/ha

T_4 : Untreated control

The experiments run twice were concluded at 150 days after transplanting (DAT) and the plants were removed with intact roots system and washed free of soil. Observations on plant growth parameters, gall index, nematode population in soil and root were made (Plate 3).

3.3.1.1.1. Nematode gall index

In the above experiments the incidence of root knot nematode in terms of gall index were made based on 1-5 scale rating (Heald *et al*., 1989).

Galling / plant (%)	Gall index
1-10	1
11-25	2
26-50	3
51-75	4
> 75	5

3.3.1.2. Management of fungal diseases in different crops

3.3.1.2.1. *F. oxysporum* f.sp. *lycopersici* on tomato

3.3.1.2.1.1. Multiplication of test fungus

The test fungus *F. oxysporum* f.sp. *lycopersici* spared by the Department of Plant Pathology, TNAU, Coimbatore was multiplied on sand maize medium as follows (Riker and Riker, 1936).

Sterilized sand and corn maize seeds were mixed in the ratio of 9:1 and sprinkled with water upto 50 per cent moisture, filled in a 500 g autoclavable polythene bags and autoclaved at 121^{o}C (30 lbs) for 20 min thrice. Then the fungus *F. oxysporum* f.sp. *lycopersici* was inoculated and incubated at 28±2^{o}C for 25 days. On multiplication of the fungus on sand maize medium the spore load was assessed using haemocytometer as 2x10^{6}cfu/g and used for the present study.

3.3.1.2.1.2. Management of *F. oxysporum* f.sp. *lycopersici*

Experiments were conducted twice on tomato (Var: PKM1) to study the influence of green synthesised AgNPs in comparison with biocontrol agent and chemical pesticide as follows for the management of Fusarium wilt disease caused by *F. oxysporum* f. sp. *lycopersici*. All the treatments were replicated five times in CRD (Plate 4).

Details of treatments

T_1 : Silver nanoparticles at 0.1%

T_2 : *P. fluorescens* @ 2 .5kg/ha

T_3 : Carbendazim at 0.1%

T_4 : Untreated control

On transplantation of four weeks old healthy tomato seedlings @ 2 seedlings /pot filled with 5 kg stream sterilized pot mixture prepared as above, the seedlings established were thinned to one/pot at 7 DAT followed by imposing treatments. The test pathogen *F. oxysporum* f.sp. *lycopersici* was inoculated @ 150 g per pot around the root zone of tomato at 15 DAT.

On termination of experiments made at 150 DAT the plants were uprooted from each pot and observations were made on per cent disease incidence (PDI) and plant growth characters.

3.3.1.2.2. *M. phaseolina* on blackgram

3.3.1.2.2.1. Multiplication of test fungus

The test fungus *M. phaseolina* obtained from the Department of Plant Pathology, TNAU, Coimbatore was multiplied on sand maize medium with slight modifications as

follows (Riker and Riker, 1936). Sterilized sand and corn maize seeds were mixed in the ratio of 9:1 and sprinkled with water upto 50 per cent moisture, filled in a 500 g autoclavable polythene bags and autoclaved at 121^{o}C (30 lbs) for 20 min four times. Then the fungus *M. phaseolina* was inoculated and incubated at 28±2^{o}C for 15 days. Finally the spore load of *M. phaseolina* multiplied on sand maize medium was assessed using haemocytometer as 2x10^{6}cfu/g and used for the present study.

3.3.1.2.2.2. Management of *M. phaseolina*

Experiments were conducted twice for the management of charcoal rot caused by *M. phaseolina* in blackgram (Plate 5). The treatments evaluated and methodology followed are same as given in the section 3.3.1.2.1.2.

The seeds of blackgram (5 seeds/pot) were sown in 5 kg earthen pots filled with sterilized pot mixture and the established seedlings were thinned @ one seedling/pot at 7 DAS before imposing treatments as mentioned in the above section. Then the test pathogen of *M. phaseolina* multiplied on sand maize medium was inoculated @ 100 g per pot around the root zone.

On termination of experiments made at 75 DAS the plants were uprooted from each pot and observations were made on (PDI) and plant growth characters. All the treatments were replicated five times in CRD.

3.3.1.2.2.3. Per cent disease incidence (PDI)

The per cent disease incidence was calculated as follows**.**

$$PDI = \frac{\text{No. of infected plants}}{\text{No. of healthy plants}} \times 100$$

3.3.1.3. Management of bacterial disease caused by *R. solanacearum* on brinjal

The test bacterial pathogen, *R. solanacearum* obtained from the Department of Agricultural Microbiology, TNAU, Coimbatore was multiplied as follows and used in the present study.

3.3.1.3.1. Multiplication of *R. solanacearum*

The cell suspension of *R. solanacearum* was prepared in the sterile distilled water to a concentration of 10^{8} cfu / ml and one ml of the bacterial cell suspension prepared was

mixed with 100 ml nutrient broth (NB) and kept in rotary shaker run at 150 rpm for 24 hr. The NB of 24 hr old bacterial culture maintained at 28±2ºC (Winstead and Kelman, 1952) was used for inoculation purpose in the present study.

3.3.1.3.2. Management of bacterial wilt caused by *R. solanacearum*

Two experiments were conducted on brinjal (Var: CO2) for the management of wilt causing bacterial pathogen of *R. solanacearum* using green synthesised AgNPs in comparison with bioagent and chemical pesticide as mentioned below.

T_1 : Silver nanoparticles at 0.1%

T_2 : *P. fluorescens* @ 2 .5kg/ha

T_3 : Streptomycin sulphate at 500 ppm

T_4 : Untreated control

The bacterium *R. solanacearum* was inoculated by root inoculation technique (Winstead and Kelman, 1952) to 30 days old brinjal plants and transplanted in 5 kg earthen pots containing sterilized pot mixture as mentioned above followed by imposing treatments. All the treatments were replicated five times in CRD (Plate 6).

The experiments were concluded at 150 DAT and observations on per cent wilt incidence was recorded in addition to plant growth characters including fruit yield.

3.3.1.3.3. Assessment of per cent wilt

$$\text{Per cent wilt} = \frac{\text{Number of plants/branches wilted}}{\text{Total number of plants}} \times 100$$

3.3.1.4. Management of nematode fungal/bacterial disease complex in different crops

3.3.1.4.1. Nematode fungal disease complex

3.3.1.4.1.1. In tomato

Experiments were conducted on tomato (Var: PKM1) for the effectiveness of green synthesised AgNPs and to compare its effect with bioagent and chemical pesticides in the management of nematode fungal disease complex involving *M. incognita* and *F. oxysporum* f.sp. *lycopersici*. The following are the treatments replicated five times in CRD (Plate 7).

Details of treatments

T_1 : Silver nanoparticles at 0.1%

T_2 : *P. fluorescens* @ 2 .5kg/ha

T_3 : Carbofuran 3G @ 1Kg a.i/ha + Carbendazim at 0.1 %

T_4 : Untreated control

The healthy tomato seedlings of four weeks old were transplanted in earthen pots (5 kg capacity) filled with sterilized pot mixture as mentioned earlier. On thinning of seedlings at the rate of one seedling/pot at 7 DAT the above treatments were applied around the root zone followed by the simultaneous inoculation of *M. incognita* ($1J_2$/g soil) and *F. oxysporum* f.sp. *lycopersici* (150 g/pot).

On termination of experiments made at 150 DAT the plants were uprooted from each pot and indexed for disease severity of nematodes in terms of root gall index and disease incidence in terms of per cent disease incidence (PDI). Besides observations were made on nematode populations in soil and root and plant growth characters including fruit yield.

3.3.1.4.1.2. In blackgram

Pot culture experiments were conducted on black gram (Var: CO5) for the management of nematode fungal disease complex involving *M. incognita* and *M. phaseolina* using a set of treatments and methodology mentioned in the previous section (Plate 8).

The seeds of blackgram (5 seeds /pot) were sown in 2.5 kg earthen pots filled with sterilized pot mixture. On germination the seedlings were thinned to one per pot at 7 DAS and simultaneously inoculated with freshly hatched J_2 of *M. incognita* ($1J_2$/g soil) and *M. phaseolina* (100 g/ pot) multiplied on sand maize medium in and around the root zone to a depth of 2-3cm.

On termination of experiments made at 75 DAS the plants were uprooted from each pot and indexed for disease severity of nematodes in terms of gall index and their populations in soil and root and PDI besides plant growth characters. All the treatments were replicated five times in CRD.

3.3.1.4.2. Nematode bacterial disease complex

3.3.1.4.2.1. In brinjal

Pot culture experiments were conducted under glasshouse conditions on brinjal (Var: CO2) for the management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* for the effectiveness of green synthesised AgNPs in comparison with bioagent and chemical pesticides. The methodology used for the management of nematode fungal disease complex was followed in the present experiment with the following treatments replicated five times in CRD.

T_1 : Silver nanoparticles at 0.1%

T_2 : *P. fluorescens* @ 2 .5kg/ha

T_3 : Carbofuran 3G @ 1Kg a.i/ha + Streptomycin sulphate at 500ppm

T_4 : Untreated control

The healthy seedlings of four weeks old brinjal bacterized by root inoculation technique (Winstead and Kelman, 1952) with *R. solanacearum* multiplied on NA broth were transplanted at 2 seedlings/pot (5 kg capacity) containing sterilized pot mixture.

On establishment of seedlings thinning was carried out at 7 DAT to maintain uniform growth of seedlings @ 1/pot. Then J_2 of *M. incognita* (1/g soil) obtained from pure culture were inoculated around the root zone of brinjal followed by the application of treatments.

In the experiments concluded at 150 DAT observations on per cent wilt and gall index were recorded in addition to nematode population both in soil and root and plant growth characters (Plate 9).

3.3.1.4.3. Observations made on plant growth characters and nematode population /incidence

In the above experiments conducted for the management of root knot nematode, fungal/bacterial plant pathogens and their disease complexes under glasshouse conditions observations on plant growth characters and nematode population/incidence were recorded as follows.

3.3.1.4.3.1. Plant growth characters

3.3.1.4.3.1.1. Shoot height

The shoot height was recorded by measuring the above ground height of test plants and expressed in cm.

3.3.1.4.3.1.2. Shoot weight

The shoot weight was recorded by measuring the above ground weight of test plants and expressed in g.

3.3.1.4.3.1.2. Root length

The root length was recorded by measuring the below ground length of test plants and expressed in cm.

3.3.1.4.3.1.3. Root weight

The root weight was recorded by measuring the below ground weight of test plants and expressed in g.

3.3.1.4.3.2. Nematode population/ incidence

3.3.1.4.3.2.1. In soil

The *M. incognita* population in soil was recorded at the time of termination of the experiment by drawing a composite sample of 250 cc soil from each pot. The samples were processed for the extraction of nematodes using the method of Cobb's sieving and decanting followed by modified Baermann's funnel technique and the population of nematode was counted and expressed in number per 250 cc soil (Schindler, 1961).

3.3.1.4.3.2.2. In root

In a set of composite root sample of 5 g drawn from each plant one set was used for extracting the J_2 of *M. incognita* using warring blender and another set of root samples was stained with acid fuschsin lactophenol followed by destaining with plain lactophenol for counting number of females and number of females with egg masses of *M. incognita* (Mc Beth *et al*., 1941). The number of eggs /egg mass were separated by

dispersion of eggs with 2 per cent sodium hypochlorite and counted under microscope. The observations made were expressed in number per unit weight of root.

3.3.2. In plant growth chamber

The results of experiments conducted for the influence of green synthesised AgNPs under controlled glasshouse conditions was reconfirmed by carrying out experiments in the plant growth chamber conditions with temperature of 25^{o}C, RH of 75 per cent and light intensity of 12,000 lux for nine hours.

In these experiments tubular size mud pots of 1 kg capacity filled with sterilized pot mixture were used to raise the test crops of tomato, brinjal and blackgram. The inoculation of root knot nematode *M. incognita;* the fungal pathogens *viz.*, *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina*; bacterial pathogen of *R. solanacearum* and application of green synthesised AgNPs were made as mentioned in the earlier experiments conducted under glasshouse conditions. All the experiments were concluded at 45 DAS/DAT (Plate 10).

In these experiments conducted on different crops untreated and treated with green synthesised AgNPs in plant growth chamber without replication/design for the management of plant pathogens and their disease complex, the direct values/count on plant pathogens, plant growth parameters etc. were used to draw the conclusion of the study.

3.4. Impact of green synthesized AgNPs on beneficial soil microorganisms

In order to ascertain illeffects of the green synthesized AgNPs if any against in the experiments were conducted *in vitro* on beneficial entomopathogenic nematode (EPN), fungal and bacterial bioagents.

3.4.1. EPN (*Steinernema glaseri)*

An experiment was conducted under laboratory conditions (28±2^{o}C) to assess the illeffects of green synthesized AgNPs on *S. glaseri* (Kucharska *et al*., 2009) provided by the Department of Nematology, TNAU, Coimbatore.

The different concentrations of green synthesised AgNPs were sonicated for

45 min in ultrasonicator to disperse the nanoparticles uniformly in double distilled water. Five ml of each concentrations was transferred to sterilized Petri dishes (5 cm dia) and the dauer infective stage (DIJ_3) *S. glaseri* were released @100 DIJ_3 / Petri dish to study the effect of AgNPs on *in vitro* test. All the concentrations as treatment and untreated control were replicated four times in CRD.

Observations were recorded on surviving ability / mortality of *S. glaseri* (DIJ_3) at 24 hr interval for a week and the per cent mortality of juveniles was worked out.

3.4.2. Fungal biocontrol agents

The required fungal culture of *Trichoderma viride* and *Paecilomyces lilacinus* received from the Department of Plant Pathology, TNAU, Coimbatore and Horticultural Research Station, Ooty were used in the present study.

The experimental procedure followed for the antifungal effect of green synthesized AgNPs as outlined in the section 3.2.3.1 was followed for studying the adverse effect of AgNPs on beneficial fungi *viz., T. viride* and *P. lilacinus* with the following concentrations of green synthesised AgNPs.

T_1 : 0.010%

T_2 : 0.025%

T_3 : 0.050%

T_4 : 0.075%

T_5 : 0.100%

T_6 : Untreated control

3.4.3. Bacterial biocontrol agents

In vitro studies were conducted to assess the illeffects of AgNPs if any against the bacterial biocontrol agents *viz., P. fluorescens* and *Bacillus subtilis*. The test culture of above bacteria were obtained from the Department of Nematology and Plant Pathology, TNAU, Coimbatore.

The study was conducted by agar well diffusion method using NA medium amended/unamended with different concentrations of the green synthesised AgNPs as follows.

The NA medium prepared was autoclaved at 121^{o}C (30 lbs) for 20 min and the culture of *P. fluorescens* and *B. subtilis* were added separately into the medium (5:100 ml), poured into the Petri plates and made to solidify under aseptic condition. Wells were formed by cutting at four places at 1cm distance away from the periphery and at centre of Petri dish using cork borer with 9 mm dia and the different concentrations of green synthesised AgNPs *viz.* 0.025, 0.050, 0.075, 0.1 per cent were added in the well of Petri plates. The Perti plates without amending AgNPs were served as untreated control. All the treatments were replicated four times in CRD. The plates were incubated at 25 ±2°C. The growth of *P. fluorescens* and *B. subtilis* and the zone of inhibition was observed and measured with scale 24 hr after inoculation and expressed in mm.

3.5. Data analysis

The data generated in the present study were subjected to statistical analysis using the statistical software Design Expert version 7.1.6, AGRES and Excel-2013 (Panse and Sukhamte, 1978).

The data were analysed and the effect of treatments were compared at $p \leq 0.05$ level of significance using the critical difference (CD) test which was performed by Excel-2013.

CHAPTER IV

EXPERIMENTAL RESULTS

Results obtained from a series of laboratory studies attempted to synthesis and characterize green synthesized silver nanoparticles using plant extracts; their effect on root knot nematode, fungal and bacterial plant pathogens, nematode fungal/ bacterial disease complex *in vitro*, controlled glasshouse conditions and in plant growth chamber besides their effect on beneficial microorganisms are presented in this chapter.

4.1. Green synthesis of silver nanoparticles

The green synthesis of silver nanoparticles (AgNPs) was successful using plant extracts of *Tridax procumbens*, *Euphorbia hirta* and *Azardirachta indica*.

4.2. Characterization of green synthesized AgNPs

The synthesized AgNPs was characterized for its size, shape, stability, nature etc. using Particle size analyzer, UV-Vis Spectroscopy, Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) with Energy Dispersive X-Ray Spectroscopy (EDAX), X-Ray Diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FTIR).

4.2.1. Particle size analyzer

The particle size analyzer used to estimate the average particle size of synthesized AgNPs revealed the particle size of 78.9, 87.6 and 40.4 nm with plant extracts of *T. procumbens* (Fig. 2), *E. hirta* (Fig. 3) and *A. indica* (Fig. 4) respectively used for the synthesis of AgNPs in the present study.

The Zeta potential was used to measure the physical stability and colloidal systems of synthesized AgNPs was registered as -41.7, -27.9 and -37.2 mV with *T. procumbens* (Fig. 5), *E. hirta* (Fig. 6) and *A. indica* (Fig. 7) respectively. The Zeta potential distributed with wide range of -41.7 mV indicated the highly stable in nature of AgNPs synthesized using *T. procumbans*.

4.2.2. UV- Vis Spectroscopy

The synthesized AgNPs initially showed yellowish color in aqueous solution due to excitation of surface plasma resonance of AgNPs. Then the color of the solution changed from yellow to brown on addition of plant extracts to silver nitrate. The color change indicated the formation of AgNPs. Further, it was confirmed with the wavelength of 435, 444 and 400 nm respectively recorded with the plant extracts of *T. procumbens* (Fig. 8), *E. hirta* (Fig. 9) and *A. indica* (Fig. 10) respectively. Since the registered wavelength falls within the prescribed range of 430-460 nm confirming the formation of AgNPs.

4.2.3. SEM

The morphology of the synthesized AgNPs using *T. procumbens* (Plate 11), *E. hirta* (Plate 12) and *A. indica* (Plate 13) respectively were observed through SEM. The observations revealed that the AgNPs were spherical in their shape and agglomerated.

4.2.3.1. EDAX

The EDAX profile showed the highest silver elemental composition of synthesized AgNPs and it was highest (97.91 %) with *T. procumbens.* It was followed by *E. hirta* (86.3 %) and *A. indica* (78.12 %) respectively.

4.2.4. TEM

The observations made through the TEM revealed the spherical shape of AgNPs with size ranging from 15.5 to 31.8 nm, 35 to 49 nm and 26.7 to 43.2 nm in respect of *T. procumbens* (Plate 14), *E. hirta* (Plate 15) and *A. indica* (Plate 16) respectively. Therefore, it is inferred that the AgNPs size was lowest with *T. procumbens* among the three plant extracts used for the green synthesis of AgNPs.

4.2.5. XRD

The X-Ray Diffraction (XRD) pattern profile of all the three plants mediated synthesized AgNPs samples quite consistently matched well with the standard nano Ag patterns.

The XRD patterns recorded in the 2θ range of 10-80° with the synthesized AgNPs indicated the crystalline nature of AgNPs by showing strong diffraction peaks at 28.60^0, 32.50^0, 38.38^0, 44.56^0, 46.42^0, 55.57^0, 64.78^0 and 77.02^0 for *T. procumbens* (Fig. 11), 27.65^0, 31.98^0, 37.62^0, 45.13^0, 53.22^0, 55.32^0, 61.30^0 and 71.66^0 for *E. hirta* (Fig. 12) and 27.74^0, 32.05^0, 26.39^0, 45.17^0, 52.96^0, 55.24^0, 61.32^0 and 71.70^0 for *A. indica* (Fig. 13).

The *T. procumbans* mediated synthesis of AgNPs showed the maxima at $2\theta=32.50^0$ which corresponding to the **d** spacing of 2.75 Å, *E. hirta* showed the maxima at 2θ=31.98° with corresponding **d** spacing of 2.76Å and *A. indica* also showed the maxima at 2θ=32.05° with corresponding **d** spacing of 2.75Å. All the above plant extracts exhibited the diffraction pattern which is characteristic to indicate crystalline nature of AgNPs.

The XRD data confirmed that the AgNPs was crystalline in nature as the values matched well with the standard reference of Joint Committee on Powder Diffraction Standards (JCPDS Card No.04-0783) of silver nanoparticles. The sharp and clearly visible peaks of XRD profile confirmed that the particles were crystalline.

4.2.6. FTIR Spectroscopy

The FTIR spectral analysis showed the presence of possible functional groups of phytochemicals in plant extracts used for the synthesis of AgNPs as per standard reference of Infrared (IR) charts. The synthesized AgNPs using leaf extracts of *T. procumbens* (Fig. 14) and *E. hirta* (Fig. 15) exhibited intense absorption peaks at 867.92, 1639.41 and 3357.90 cm^{-1} corresponding to strong stretching of C–H aromatics. The medium stretching band indicated the presence of N-H primary and secondary amides and the broad band denoted as -OH stretching indicated the presence of alcohols and phenols as functional groups of biomolecules of the plant extracts. Similarly *A. indica* (Fig. 16) showed the peak at 1641.34, 2360.75 and 3359.83 cm^{-1} with corresponding medium stretch of –C=C–alkenes. The medium and broad stretching of H–C=O: C–H aldehydes and -OH alcohols and phenols indicated that biomolecules present in the extracts acted as reducing, capping and stabilizing agents for the development of AgNPs.

4.3. Evaluation of the AgNPs for their antimicrobial properties

All the three plants *viz., T. procumbens*, *E. hirta* and *A. indica* tested were found to be suitable for the green synthesis of AgNPs in the present study. However it is evident from the Table 5 that the synthesized AgNPs using *T. procumbens* were having smallest average particle size (15.5 to 31.8 nm), possessing the highest silver elemental composition (97.91 %) due to its capability of synthesizing silver at optimum peak of 435 nm and crystalline in nature Hence *T. procumbens* is considered as more desirable plant source compared to the other plants tested in the present study.

Therefore, the AgNPs synthesized using *T. procumbens* was advanced for testing its antimicrobial properties and other studies.

4.3.1. Antinemic properties

The *T. procumbens* mediated synthesized AgNPs possessing desirable characters of AgNPs was tested on root knot nematode *Meloidogyne incognita* for its ovicidal and larvicidal effect; and its influence on nematode behavior like attraction and root penetration in tomato.

4.3.1.1. Influence on eggs of *M. incognita*

The AgNPs synthesized using *T. procumbens* had profound effect to inhibit the egg hatching of *M. incognita* and its effect was found to be positively correlated with its concentrations and period of exposure. The ovicidal effect of *T. procumbens* mediated synthesized AgNPs was differing significantly among concentrations and time of exposure.

The inhibition in egg hatching was highest (91.49 %) at the highest concentration of 0.0005 per cent of AgNPs at 7 days after inoculation. The per cent inhibition in egg hatching of *M. incognita* was ranged from 49.77 to 87.35 in other concentrations of 0.00001 to 0.0001 per cent and time of exposure of 1 to 7 days (Table 6).

4.3.1.2. Influence on juveniles of *M. incognita*

All the concentrations of AgNPs using *T. procumbens* caused mortality of juveniles of *M. incognita* at all the period of exposure compared to untreated control and

was differing significantly with each other. The effect of AgNPs had direct relationship between rate of mortality of J_2 of *M. incognita* and concentrations / period of exposure.

There was cent per cent mortality of juveniles of *M. incognita* at higher concentrations of 0.0005 and 0.0001 per cent of the green synthesized AgNPs within the shortest period of exposure of 24 hr and the trend was continued at all the period of exposure. Similarly the cent per cent mortality of juveniles of *M. incognita* was also caused by the lower concentration of 0.00005 but after sixth day of exposure. The mortality of *M. incognita* was ranging from 20.35 to 36.82 per cent by the lowest concentration of 0.00001 per cent of AgNPs using *T. procumbens* (Table 7).

4.3.1.3. Influence of AgNPs on attraction and root penetration of *M. incognita* in tomato

The AgNPs possessing ovicidal and larvicidal effect on *M. incongita* was tested for its influence on attraction and root penetration in tomato.

4.3.1.3.1. Attraction of *M. incognita*

There was considerable reduction in the attraction of juveniles (J_2) of *M. incognita* in tomato roots treated with the AgNPs at all the concentrations and period of exposure. It was significant compared to untreated control. The rate of attraction of J_2 of *M. incognita* was inversely proportional to the concentrations of AgNPs. Further, the per cent reduction in the attraction of *M. incognita* (J_2) towards the tomato roots treated with AgNPs was reduced with increase in the period of exposure of 24 to 120 hr and all the concentrations of 0.0005 to 0.00001 per cent tested in the present study.

The highest reduction (94.67 %) in the attraction of J_2 of M. *incognita* was recorded by 0.0005 per cent concentration and it was ranging from 59.15 to 91.14 per cent in other concentrations of 0.00001 to 0.0001 of the AgNPs irrespective of period of exposure (Table 8).

4.3.1.3.2. Root penetration of *M. incognita*

The experimental results revealed that there was significant reduction in the rate of root penetration of J_2 of *M. incognita* in tomato at all the concentrations and period of exposure to AgNPs. A positive correlation exists between the per cent root penetration of

M. incognita (J_2) with the concentration of green synthesized AgNPs. However, the per cent root penetration of *M. incognita* in tomato was found to be reduced with increase in the period of exposure from 1 to 7 days.

The attraction of *M. incognita* (J_2) was highest (79.76 to 90.16 %) towards root treated with highest concentration of 0.0005 per cent of AgNPs at all the period of exposure. It was lowest (42.02 to 64.77 %) at lowest concentration of 0.00001 per cent of the AgNPs (Table 9).

4.3.2. Antifungal properties

4.3.2.1. Against *Fusarium oxysporum* f.sp. *lycopersici*

The antifungal property of AgNPs was evaluated *in vitro* against *F. oxysporum* f.sp. *lycopersici.* The results indicated significant inhibitory effect on the mycelial growth of the test fungus by all the concentrations of 0.001 to 0.1 per cent and it was ranging from 96.67 to 81.11 per cent over untreated control. The antifungal effect of different concentrations was differing significantly from each other (Table 10).

The highest concentration of 0.1 per cent of AgNPs caused maximum (96.67 %) reduction in the radial mycelial growth of *F. oxysporum* f.sp. *lycopersici*. The present study established direct relationship between suppression in the growth of *F. oxysporum* f.sp. *lycopersici* and the concentrations of AgNPs (Plate 17).

4.3.2.2. Against *Macrophomina phaseolina*

Different concentrations of AgNPs (0.001 to 0.1 %) were evaluated *in vitro* against *M. phaseolina* to determine the antifungal property of AgNPs using PDA medium (Plate 18). The results of the study revealed that all the concentrations of AgNPs were found to arrest the mycelial growth of the test fungus at varying level of 82.63 to 98.88 per cent over untreated control and the effect was significant among each other. The highest (98.88 %) reduction in the radial mycelial growth of *M. phaseolina* was registered at 0.1 per cent of AgNPs. There was positive correlation between suppression in the growth of *M. phaseolina* and the concentrations of AgNPs (Table 11).

4.3.3. Antibacterial properties

4.3.3.1. Against *Ralstonia solanacearum*

The AgNPs was proved to be antibacterial *in vitro* against *R. solanacearum*. In the present study the lower concentrations of 0.001 and 0.005 per cent had no inhibitory effect over the test bacterium. However, the higher concentrations of 0.01 to 0.1 per cent of AgNPs was found to be inhibitory to the *R. solanacearum* as the extent of zone of inhibition was higher (95.03 to 97.54 %) over untreated control (Table 12).

The inhibitory effect of AgNPs over *R. solanacearum* was observed to be concentration dependent in the present study (Plate 19).

4.3.3.2. Against *Xanthomonas oryzae* pv *oryzae*

As mentioned earlier the lower concentrations of 0.001 and 0.005 per cent of AgNPs did not show any antibacterial effect over *X. oryzae* pv *oryzae* (Table 12). However, the higher concentrations of 0.01 and above AgNPs proved to be antibacterial against the test bacterium. The highest zone of inhibition (97.30 %) was registered at 0.1 per cent concentration of green synthesized AgNPs (Plate 20).

The antibacterial effect of different concentrations of the AgNPs was found to be concentrations dependent as observed earlier.

4.3.3.3. Morphological changes caused by the green synthesized AgNPs on test pathogens

4.3.3.3.1. Root knot nematode

SEM studies on the ultrastructure of root knot nematode eggs on exposure to AgNPs revealed that the eggs were completely deformed. Healthy eggs were intact whereas the treated eggs had lost their structural integrity (Plate 21 and 22). Similarly the dead juveniles of root knot nematode on exposure to the most effective concentration of 0.0005 per cent of AgNPs appeared to be deformed with rupture of cell wall (Plate 23).

4.3.3.3.2. Fungi

4.3.3.3.2.1. *F. oxysporum f.sp. lycopersici*

The hyphal surface of the fungus was observed to be uneven with blunt tip and also found to be crinkled and shriveled when exposed to 0.1 per cent concentration of AgNPs (Plate 24).

4.3.3.3.2.2. *M. phaseolina*

As above the fungal hyphae were uneven in its length and width; dull in colour, crinkled and shriveled with reduction in the formation of sclereospore (Plate 25).

4.4. Management of plant pathogens using AgNPs in different crops

4.4.1. Under glasshouse conditions

4.4.1.1. Influence of AgNPs on *M. incognita* in tomato

The results of observations made on the nematode population/incidence and yield attributes of tomato in the experiments conducted twice under glasshouse conditions to evaluate the effect of AgNPs in comparison with bacterial bioagent and chemical nematicide are furnished in Tables 13 and 14.

4.4.1.1.1. *M. incognita* population / incidence

The experimental data revealed the promising significant effect of AgNPs over the bioagent, *Pseudomonas fluorescens* and chemical nematicide carbofuran on the suppression of the root knot nematode population and to reduce its disease severity in terms of gall index on tomato (Table 13).

4.4.1.1.1.1. Nematode population in soil

The AgNPs at 0.0005 per cent was highly effective to reduce the nematode population by 88.16 per cent compared to *P. fluorescens* (79.49 %) and carbofuran (75.77 %). The difference in the degree of reduction in nematode population was significant among the treatments (Table 13).

4.4.1.1.1.2. Nematode population in root

The above results was also observed with respect to reduction in *M. incognita* population in root by the soil application of AgNPs at 0.0005 per cent in tomato and it was higher (92.79 %) than the effect of *P. fluorescens* (79.77 %) and carbofuran (66.10 %). The effect of AgNPs in the suppression of *M. incognita* population in roots of tomato was significantly superior over *P. fluorescens*, carbofuran and untreated control (Table 13).

4.4.1.1.1.3. Number of egg masses/g root

The *T. procumbens* mediated AgNPs evaluated at 0.0005 per cent concentration registered the lowest number of females with egg mass (2.68) with the highest per cent reduction of 79.80 over untreated control. The effect of AgNPs was followed by *P. fluorescens* (40.52 %) and carbofuran (15.15 %) and their effect was differing significantly from each other and untreated control registered the highest number of females with egg mass (13.27) (Table 13).

4.4.1.1.1.4. Eggs/egg mass

The experimental data furnished in Table 13 indicated that the AgNPs, *P. fluorescens* and carbofuran had significant effect to reduce the fecundity of *M. incognita* over untreated control (290.33). Among them the AgNPs was ranking first (65.14 %) and it was followed by *P. fluorescens* (61.96 %) and carbofuran (58.55 %) in reducing the number of eggs/egg mass of root knot nematode.

4.4.1.1.1.5. Gall index

The lowest gall index of 0.2 was registered by the *T. procumbens* mediated AgNPs as against 5 in untreated control and it was lower than the gall index recorded by the plants treated with *P. fluorescens* (1) and carbofuran (3).

Therefore it is inferred that the AgNPs capable of reducing root knot nematode population / fecundity resulted in the lowest nematode disease incidence as measured by gall index.

4.4.1.1.2. Plant growth parameters

Observations recorded on yield attributes in the experiment conducted under glasshouse conditions to evaluate the influence of AgNPs for the management of *M. incognita* in comparison with other standard practices of using *P. fluorescens* and carbofuran in tomato are furnished in Table 14 and Plate 26.

4.4.1.1.2.1. Shoot height

There was significant improvement in shoot height of tomato due to application of AgNPs, *P. fluorescens* and carbofuran and increase in shoot height was ranged from

13.11 to 26.99 per cent over untreated control. Among them the AgNPs had the highest effect (26.99 %) and it was followed by *P. fluorescens* (25.61 %) and carbofuran (13.11 %) and their effect was differing significantly among each other and from untreated control (Table 14).

4.4.1.1.2.2. Shoot weight

The above trend of increase was observed in shoot biomass also following the soil application of AgNPs (152.35 g), *P. fluorescens* (149.09 g) and carbofuran (140.25 g) with 13.92, 12.03 and 6.49 per cent increase over untreated control respectively. It was significantly different from each other (Table 14).

4.4.1.1.2.3. Root length

The tomato plants treated with the AgNPs at 0.0005 per cent recorded the highest root length of 37.90 cm with 26.57 per cent increase over untreated control (27.83 cm). It was followed by *P. fluorescens* (20.62 %) and carbofuran (10.94 %) and there was significant difference among each other in increasing the root length of tomato (Table 14 and Plate 27).

4.4.1.1.2.4. Root weight

There was significant increase in root weight by the application of AgNPs (23.45 %) followed by *P. fluorescens* (17.89 %) and carbofuran (12.27 %) over untreated control (23.64 g) with significant difference among each other (Table 14).

4.4.1.1.2.5. Fruit yield

There was significant increase in fruit yield followed by the soil application of AgNPs (20.54 %), *P. fluorescens* (15.65 %) and carbofuran (10.71 %) over untreated control (Table 14). Therefore, it is inferred that the AgNPs had effect to improve the yield attributes resulted in considerable increase in fruit yield of tomato.

4.4.1.2. Fungal pathogens

4.4.1.2.1. *F. oxysporum* f. sp. *lycopersici* on tomato

The Fusarium wilt incidence caused by *F. oxysporum* f. sp. *lycopersici* of tomato was considerably reduced by the AgNPs (60.52 %) when compared to bacterial bioagent,

P. fluorescens (30.00 %) and chemical fungicide, carbendazim (26.05 %) (Table 15). Therefore, it is proved that the AgNPs was in possession of antifungal property besides antinemic property as mentioned earlier.

4.4.1.2.1.1. Plant growth characters

There was improvement in growth of tomato plant consequent to the suppression of the Fusarium wilt incidence by the application of AgNPs in tomato as follows (Table 15 and Plate 28).

4.4.1.2.1.1.1. Shoot height

The increase in shoot height by the application of AgNPs, *P. fluorescens* and carbendazim was ranged from 8.76 to 21.74 per cent over untreated control. The highest shoot height of 69.91 cm was registered by the AgNPs and it was significant over *P. fluorescens* (67.53 cm), carbendazim (59.96 cm) and untreated control (54.71 cm).

4.4.1.2.1.1.2. Shoot weight

Similar trend was observed in respect of shoot weight also with highest increase by the AgNPs (21.51 %). It was followed by *P. fluorescens* (20.59 %) and carbendazim (6.37 %) with significant difference among each other (Table 15).

4.4.1.2.1.1.3. Root length

The highest root length of 36.71 cm with 29.99 per cent increase over untreated control was recorded by the AgNPs. It was significant over other inputs *viz.*, *P. fluorescens* (26.11 %) and carbendazim (11.84 %) evaluated for the management of Fusarium wilt of tomato in the present study (Table15).

4.4.1.2.1.1.4. Root weight

The green synthesized AgNPs used for the management of Fusarium wilt of tomato resulted in highest increase in root weight (25.39 g) with 26.81 per cent increase over untreated control (18.58 g) and it was significant. The effect of AgNPs in increasing the root weight of tomato was followed by *P. fluorescens* (20.01 %) and carbendazim (10.75 %) with significant difference among each other.

4.4.1.2.1.1.5. Fruit yield

There was remarkable increase in the economic fruit yield of tomato following the management of Fusarium wilt incidence using AgNPs and it was highest (14.77 %) than *P. fluorescens* (12.04 %) and carbendazim (8.73 %) used for comparison as biocontrol agent and chemical fungicide respectively. The fruit yield recorded in untreated control was 82.20 g (Table 15).

4.4.1.2.2. *M. phaseolina* on blackgram

There was considerable reduction in disease incidence caused by *M. phaseolina* by the AgNPs (50.26 %), *P. fluorescens* (44.82 %) and carbendazim (42.40 %) over untreated control with significant difference among each other (Table 16).

Therefore, the use of AgNPs was proved as an effective measure for the management of charcoal rot caused by *M. phaseolina* in blackgram compared to *P. fluorescens* and carbendazim in the present study (Plate 29).

4.4.1.2.2.1. Plant growth characters

The plant growth characters measured in terms of length and weight of shoot and root showed significant improvement among the treatments in the experiments conducted under glasshouse conditions for the management of charcoal rot of blackgram caused by *M. phaseolina* (Table 16).

4.4.1.2.2.1.1. Shoot height

The shoot height in plants treated with AgNPs was highest (37.62 cm) and it was followed by *P. fluorescens* (35.21 cm) and carbendazim (30.33 cm) and differing significantly among each other and untreated control (26.41 cm) (Table 16).

4.4.1.2.2.1.2. Shoot weight

As above the AgNPs was ranking first in increasing shoot weight (11.42 g) of blackgram with 36.25 per cent increase over untreated control (7.28 g). It was significantly higher than the effect of *P. fluorescens* (32.34 %) and carbendazim (14.35 %).

4.4.1.2.2.1.3. Root length

There was significant increase in root length (7.53 cm) with 43.69 per cent increase over untreated control (4.24 cm) by the application of AgNPs and its effect was followed by *P. fluorescens* (35.37 %) and carbendazim (23.19 %). The difference in increasing the root length of blackgram was significant among each other (Table 16).

4.4.1.2.2.1.4. Root weight

The above trend was followed by the application of AgNPs with the highest increase in root weight (5.62 g) with 72.06 per cent increase over untreated control (2.57 g). It was followed by the effect of *P. fluorescens* (60.25 %) and carbendazim (15.14 %) with significant difference among each other (Table 16).

4.4.1.2.2.1.5. Seed yield

The observations made on the seed yield revealed that the AgNPs was most effective to enhance the seed yield to the extent of 56.71 per cent by improving the plant growth parameters following the suppression of charcoal rot incidence in blackgram. Its effects was followed by *P. fluorescens* (51.61 %) and carbendazim (49.15 %) as mentioned earlier (Table 16).

4.4.1.3. Bacterial pathogens

4.4.1.3.1. *R. solanacearum* on brinjal

The experimental results revealed that the AgNPs tested against the bacterial wilt causing pathogen, *R. solanacearum* in brinjal was more effective than *P. fluorescens* and streptomycin sulphate (Plate 30). The per cent reduction in wilt incidence followed by the application of AgNPs, *P. fluorescens* and streptomycin sulphate was 56.01, 37.34 and 27.88 respectively over untreated control and differing significantly among each other (Table 17).

4.4.1.3.1.1. Plant growth parameters

4.4.1.3.1.1.1. Shoot height

The shoot height (59.33 cm) recorded by the plants treated with AgNPs was highest with 21.70 per cent increase over untreated control (50.45 cm). The increase in

shoot height was 19.41 and 12.85 per cent respectively in *P. fluorescens* and streptomycin sulphate treated plants. The difference in increasing shoot height of brinjal was significant among the treatments (Table 17).

4.4.1.3.1.1.2. Shoot weight

The above trend was noticed with significant increase in shoot weight of brinjal among the treatments in plants treated with AgNPs (15.29 %), *P. fluorescens* (12.30 %) and streptomycin sulphate (9.29 %) compared to untreated control (86.80 g).

4.4.1.3.1.1.3. Root length

The AgNPs was ranking first to increase the root length of brinjal by 28.21 per cent over untreated control (14.25 cm) and its effect was differing significantly from *P. fluorescens* (25.39 %) and streptomycin sulphate (16.81 %) (Table 17).

4.4.1.3.1.1.4. Root weight

The highest root weight of 10.25 g was recorded by the AgNPs and the per cent increase over untreated control was 29.70. Its effect was followed by *P. fluorescens* (21.90 %) and streptomycin sulphate (16.70 %) evaluated as other inputs for the management of bacterial wilt of brinjal in the present study.

4.4.1.3.1.1.5. Fruit yield

Consequent to suppression in bacterial wilt incidence and subsequent improvement in plant growth characters there was increase in fruit yield of brinjal in all the treatments. The highest fruit yield recorded by the plants treated with the AgNPs (16.96 %) was differing significantly from *P. fluorescens* (13.54 %), streptomycin sulphate (9.54 %) and untreated control (100.01 g) (Table 17).

4.4.1.4. Management of nematode disease complex in different crops

4.4.1.4.1. Nematode fungal disease complex

4.4.1.4.1.1. Tomato

The results of the experiments conducted twice under controlled glasshouse conditions for the management of nematode fungal disease complex involving

M. incognita and *F. oxysporum* f.sp. *lycopercisi* using AgNPs (0.1%) in comparison with *P. fluorescens* (2.5 kg/ha) and combined application of carbofuran (1 Kg a.i/ha) with carbendazim (0.1 %) in tomato is presented in Tables 18 and 19.

4.4.1.4.1.1.1. Nematode population

4.4.1.4.1.1.1.1. Soil

The green synthesized AgNPs had profound effect to check the root knot nematode population in soil compared to the effect of bioagent and chemical pesticides and differing significantly among each other.

There was highest reduction (90.12 %) in root knot nematode population in plants treated with the AgNPs and its effect was differing significantly from *P. fluorescens* (82.18 %) used as bioagent and combination of carbofuran and carbendazim (76.83 %) used as chemical pesticides for the management of nematode fungal disease complex of tomato (Table 18).

4.4.1.4.1.1.1.2. Root

As above the AgNPs caused the highest reduction (80.22 %) in root knot nematode population in roots of tomato. Its effect was followed by the *P. fluorescens* (53.36 %) and combination of carbofuran and carbendazim (40.79 %). The effect of above inputs used for the management of nematode fungal disease complex of tomato is differing significantly among each other and untreated control (76.86/5 g root) (Table 18).

4.4.1.4.1.1.1.3. Number of egg masses /g root

There was highest reduction in number of egg masses/g of roots over untreated control in plants treated with the AgNPs (78.99 %) and it was followed by *P. fluorescens* (48.88 %) and combined application of carbofuran with carbendazim (25.34 %) and their effect was differing significantly among each other.

4.4.1.4.1.1.1.4. Eggs/egg mass

As above the AgNPs registered the highest reduction in number of eggs/egg mass (51.40 %). The effect of AgNPs in lowering the fecundity of *M. incognita* was higher than *P. fluorescens* (49.97 %) and combined application of carbofuran and carbendazim

(45.99 %) (Table 18). The effect of AgNPs was significantly higher than the effect of bioagent and chemical pesticides in this regard.

4.4.1.4.1.1.1.5. Gall index

The lowest gall index of 0.2 was recorded in plants treated with AgNPs. The effect of AgNPs was significantly higher than the effect of *P. fluorescens* (2), chemical pesticides (3) and untreated control (5) in reducing root knot nematode disease severity in terms of gall index in tomato (Table 18).

4.4.1.4.1.1.2. *Fusarium* wilt incidence

The per cent disease incidence of Fusarium wilt of tomato declined by the AgNPs (25.95), *P. fluorescens* (35.25) and combination of chemical pesticides carbofuran and carbendazim (45.10) when compared to untreated control (68.35) and their effect differing significantly among each other (Table 18).

4.4.1.4.1.1.3. Plant growth characters

4.4.1.4.1.1.3.1. Shoot height

There was significant increase in shoot height due to suppression of nematode fungal disease complex by the different inputs comprising of nanoparticles, bioagent and chemical pesticides compared to untreated control (53.15 cm) in tomato (Plate 31).

The per cent increase in shoot height was highest in plants treated with AgNPs (21.19%) and it was differed significantly from *P. fluorescens* (19.04 %) and combination of chemical pesticides with carbofuran and carbendazim (13.31 %) (Table 19).

4.4.1.4.1.1.3.2. Shoot weight

The above trend was maintained in shoot weight also by different inputs. The highest per cent increase of 38.87 over untreated control in shoot weight was achieved by the AgNPs and it was significantly differing from *P. fluorescens* (36.87 %) and combination of chemical pesticides with carbofuran and carbendazim (31.66 %).

4.4.1.4.1.1.3.3. Root length

In the per cent increase in root length ranging from 12.23 to 26.00 over untreated control by different inputs evaluated in the present study, the AgNPs was ranked first with 32.16 cm (Table 19).

4.4.1.4.1.1.3.4. Root weight

The highest root weight of 27.36 g was registered by the AgNPs with 34.21 per cent increase over untreated control (18.00 g). The effect of AgNPs was differing significantly from the bioagent (29.49 %) and chemical pesticides (15.45 %) evaluated in the present study for the management of nematode fungal disease complex of tomato (Table 19).

4.4.1.4.1.1.3.5. Fruit yield

The green synthesized AgNPs found to be most effective for the management of nematode fungal disease complex and to improve the plant growth parameters resulted with increase in fruit yield by 13.47 per cent over untreated control. Its effect was significantly higher than the effect of *P. fluorescens* (11.60 %) and combination of nematicide carbofuran and fungicide carbendazim (8.37 %) in this regard.

Therefore it is concluded that the AgNPs possessing all the desirable attributes of AgNPs was effective for the management of root knot nematode *M. incognita*, wilt causing fungus *F. oxysporum* f.sp. *lycopersici* individually and its disease complex (Table 19).

4.4.1.4.1.2. Blackgram

The results of the experiments conducted twice under controlled glasshouse conditions to evaluate the effect of AgNPs (0.1%) in comparison with *P. fluorescens* (2.5kg/ha) and carbofuran (1Kg a.i/ha) with carbendazim (0.1 %) for the management of nematode fungal disease complex involving *M. incognita* and *M. phaseolina* in blackgram is presented in Tables 20 and 21.

4.4.1.4.1.2.1. Nematode population

4.4.1.4.1.2.1.1. Soil

The AgNPs causing the highest (85.29 %) reduction in root knot nematode population in soil was regarded as the most effective measure in the present study.

The per cent reduction in root knot nematode population by the other treatments *viz.*, *P. fluorescens* (77.59) and combination of pesticides, carbofuran and carbendazim (75.85) was lower than the effect of AgNPs with significant difference among each other (Table 20).

4.4.1.4.1.2.1.2. Root

The above trend was observed in *M. incognita* population present in root also with the highest per cent reduction by AgNPs. The effect of this treatment was differing significantly from untreated control as well as *P. fluorescens* (44.68 %), chemical pesticides (37.68 %) evaluated in the present study for the management of nematode fungal disease complex of blackgram.

4.4.1.4.1.2.1.3. Number of females with egg mass/g root

As observed earlier the AgNPs registered the lowest number of females with egg mass with the highest per cent reduction of 73.59 over untreated control. Its effect was followed by *P. fluorescens* (60.30 %) and chemical pesticides (51.45 %) with significant difference among each other (Table 20).

4.4.1.4.1.2.1.4. Number of eggs / egg mass

With regard to eggs / egg mass also the highest per cent reduction (34.98) in fecundity over untreated control was observed with AgNPs. The bioagent, *P. fluorescens* (28.07 %) and combination of chemical pesticides (19.37 %) ranked as next best treatments for reducing the fecundity of *M. incognia* in blackgram (Table 20).

4.4.1.4.1.2.1.5. Gall index

The reduction in *M. incognita* population both in soil and root and fecundity at the highest level by the nematicidal effect of green synthesized AgNPs resulted in lowest gall index of 0.4. Similarly the other inputs used for the management of nematode fungal disease complex in the present study also had effect to reduce the nematode disease severity with the gall index ranging from 1 to 2 compared to untreated control (5) (Table 20).

4.4.1.4.1.2.2. Charcoal rot incidence

The highest per cent disease incidence of charcoal rot was recorded as 69.00 in untreated control. However the disease incidence was brought down significantly by different inputs used in the present study.

The results furnished in Table 20 clearly indicated that the AgNPs (31.87) was highly effective to check the charcoal rot incidence caused by *M. phaseolina* compared to biocontrol efficacy of *P. fluorescens* (38.20) and the effect of combination of chemical pesticides with carbofuran and carbendazim (44.61).

Therefore, it is concluded that the AgNPs had wide antifungal property as they were capable of inhibiting the growth and development as well as the per cent disease incidence of both fungi *viz., F. oxysporum* f.sp. *lycopersici* and *M. phaseolina*.

4.4.1.4.1.2.3. Plant growth characters

Observations made on plant growth characters followed by significant suppression in nematode fungal incidence furnished in Table 21 indicated that there was marked difference due to the effect of AgNPs, bioagent and chemical pesticides as follows (Plate 32).

4.4.1.4.1.2.3.1. Shoot height

The highest shoot height of 57.50 cm with 23.39 per cent increase over untreated control was recorded by the AgNPs. The per cent increase in shoot height in *P. fluorescens* (20.54 %) and combination of chemical pesticides (12.56 %) treated plants was lower compared to the effect of AgNPs and differing significantly among each other and untreated control (44.05 cm) (Table 21).

4.4.1.4.1.2.3.2. Shoot weight

As above the AgNPs ranked first to increase the shoot weight of blackgram with 10.34 per cent increase over untreated control and differed significantly from *P. fluorescens* (7.84 %) and chemical pesticides (3.42 %) used in the present study for the management of nematode fungal disease complex of blackgram.

4.4.1.4.1.2.3.3. Root length

The above trend of increase in plant growth characters was observed with AgNPs in respect of root length also with the highest per cent (28.20) increase over untreated control and it was similar with regard to *P. fluorescens* (23.99 %) and chemical pesticides (13.13%) as mentioned above (Table 21).

4.4.1.4.1.2.3.4. Root weight

There was significant increase in root weight in blackgram treated with AgNPs (33.81 %), *P. fluorescens* (24.96 %) and combination of chemical pesticides (13.07 %) compared to untreated control. The difference among the treatments was significant (Table 21).

4.4.1.4.1.2.3.5. Seed yield

The well pronounced effect of AgNPs in improving the plant growth characters ultimately resulted in increase in the seed yield of blackgram significantly.

The highest increase in seed yield with 47.61 per cent was registered by the AgNPs over untreated control. It was followed by *P. fluorescens* (43.37 %) and carbofuran with carbendazim (26.34 %). The difference was significant among each other (Table 21).

4.4.1.4.2. Nematode bacterial disease complex

4.4.1.4.2.1. Brinjal

The results of glasshouse experiment revealed the promising effect of AgNPs, *P. fluorescens* and chemical pesticides for the management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* in brinjal at varying level (Tables 22 and 23).

4.4.1.4.2.1.1. Nematode population/incidence

4.4.1.4.2.1.1.1. Soil

The AgNPs had highest (82.75 %) effect to check the root knot nematode population in soil. The bioagent, *P. fluorescens* (74.76 %) and combination of carbofuran

and streptomycin sulphate (71.51 %) were ranked as next best treatments for reducing the *M. incognita* population in brinjal. The difference among the treatments was significant (Table 22).

4.4.1.4.2.1.1.2. Root

The above trend was observed with the highest per cent reduction in nematode population by the AgNPs (68.12) followed by *P. fluorescens* (60.75) and combination of carbofuran and streptomycin sulphate (46.33) in *M. incognita* population of root also with significance difference among each other (Table 22).

4.4.1.4.2.1.1.3. Number of females with egg mass/g root

The highest number of females with egg mass was recorded in untreated control (8.31). Whereas the AgNPs registered the lowest number of females with egg mass (2.40) with the highest per cent reduction of 71.12 over untreated control. It was followed by *P. fluorescens* (50.42 %) and chemical pesticides (20.58 %) and their effect was differing significantly among each other (Table 22).

4.4.1.4.2.1.1.4. Number of eggs/egg mass

Although the nanoparticles, bioagent and chemical pesticides had effect to reduce the fecundity of *M. incognita* compared to untreated control (281.62) it was highest with the AgNPs using *T. procumbens* (106.86) with 62.06 per cent reduction over untreated control.

The *P. fluorescens* and the combination of carbofuran and streptomycin sulphate compared with AgNPs also had similar effect to reduce the fecundity of root knot nematode with 55.88 and 43.12 per cent decrease over untreated control. The difference in fecundity of nematode was significant among the various inputs experimented in the present study.

4.4.1.4.2.1.1.5. Gall index

The lowest gall index of 0.6 was registered by the AgNPs as against 5 in untreated control. It was also lowered in the plants treated with *P. fluorescens* (1) and carbofuran and streptomycin sulphate (2) used for comparing the effect of AgNPs in this experiment.

4.4.1.4.2.1.2. Per cent Disease Incidence

The green synthesized AgNPs (57.26 %) proved to be more effective than *P. flourescens* (44.96 %) and chemical combination of carbofuran and streptomycin sulphate (34.65 %) in checking the wilt incidence of brinjal caused by *R. solanacearum* compared to untreated control. The difference in per cent disease incidence was significant among each other (Table 22).

Therefore it is inferred that the AgNPs was effective for the management of root knot nematode, *R. solanacerarum* as well as nematode bacterial disease complex.

4.4.1.4.2.1.3. Plant biomass

4.4.1.4.2.1.3.1. Shoot height

The shoot height in plants treated with AgNPs was highest (75.68 cm) and it was followed by *P. fluorescens* (71.22 cm) and combination of carbofuran with streptomycin sulphate (67.32 cm). The difference in shoot height was significant among the treatments (Table 23 and Plate 33).

4.4.1.4.2.1.3.2. Shoot weight

As above the AgNPs was ranking first in increasing shoot weight (130.90 g) of brinjal by 7.39 per cent over untreated control (121.22 g). It was significantly higher than the effect of *P. fluorescens* (6.11 %) and carbofuran with streptomycin sulphate (3.45 %).

4.4.1.4.2.1.3.3. Root length

There was significant increase in root length (42.90 cm) with 29.14 per cent increase over untreated control (30.40 cm) by the application of AgNPs and it was followed by *P. fluorescens* (19.58 %) and chemical pesticides (10.38 %). The difference in increasing the root length of brinjal was significant among each other (Table 23).

4.4.1.4.2.1.3.4. Root weight

The above trend was followed by the application of AgNPs with the highest increase in root weight (25.85 g) over untreated control (18.39 g) and it was followed by the effect of *P. fluorescens* (24.97 %) and chemical pesticides (17.09 %) in this regard.

4.4.1.4.2.1.3.5. Fruit yield

There was remarkable increase in fruit yield of brinjal when treated with AgNPs (18.51 %). Its effect was significantly higher than the effect of *P. fluorescens* (14.83 %) and combined application of carbofuran and streptomycin sulphate (10.49 %). The difference in fruit yield recorded by different treatments was differing significantly from untreated control and among each other (Table 23).

4.4.2. Reconfirmation of the effectiveness of AgNPs in different crops for the management of plant pathogens in plant growth chamber

4.4.2.1. Tomato

4.4.2.1.1. *M. incognita*

The results of experiments conducted twice in plant growth chamber to reconfirm the effectiveness of AgNPs against *M. incognita* in tomato is furnished in Tables 24 and 25.

4.4.2.1.1.1. Nematode population/incidence

Soil and root populations of *M. incognita* were greatly reduced in plants treated with AgNPs by 72.00 and 87.09 per cent respectively over untreated control. The trend was similar in respect of number of females with egg mass (80.00 %) and number of eggs / egg mass (36.73 %). The incidence of root knot nematode in terms of gall index was also lower in AgNPs (0) compared to untreated control (1) (Table 24).

4.4.2.1.1.2. Plant growth parameters

The green synthesized AgNPs using *T. procumbens* had profound effect to check the population of *M. incognita* enhanced the shoot height (5.14 %) and weight (10.37 %); root length (34.95 %) and weight (58.54 %) over untreated control at 45 days after transplanting (DAT) (Table 25).

4.4.2.1.2. *F. oxysporum* f.sp. *lycopersici*

4.4.2.1.2.1. Wilt incidence

There was cent per cent suppression in Fusarium wilt incidence in tomato plants treated with the green synthesized AgNPs (Table 26).

4.4.2.1.2.2. Plant growth characters

The green synthesized AgNPs improved plant biomass in terms of shoot height (10.81 %) and weight (2.60 %); root length (19.23 %) and weight (41.18 %) compared to untreated control following the cent per cent suppression of wilt incidence (Table 26).

4.4.2.2. Blackgram

4.4.2.2.1. *M. phaseolina*

4.4.2.2.1.1. Charcoal rot incidence

The AgNPs was proved to be capable of suppressing the growth and development of *M. phaseolina* in blackgram completely.

4.4.2.2.1.2. Plant growth characters

The successful suppression of charcoal rot incidence following the application of AgNPs resulted in improvement in the plant growth characters of shoot height (5.22 %) and weight (3.22 %); root length (5.88 %) and weight (6.54 %) over untreated control (Table 27).

4.4.2.3. Brinjal

4.4.2.3.1. *R. solanacearum*

4.4.2.3.1.1. Bacterial wilt incidence

The AgNPs checked the wilt incidence of brinjal caused by *R. solanaearum* by cent per cent.

4.4.2.3.1.2. Plant growth characters

The present study proving the bactericidal effect of AgNPs enhanced the yield attributes of brinjal in terms of shoot height (8.57 %) and weight (5.00 %); root length (8.33 %) and weight (0.53 %) over untreated control in plant growth chamber at 45 DAT (Table 28).

4.4.2.4. Nematode fungal disease complex

4.4.2.4.1. Tomato

4.4.2.4.1.1. Nematode population and Per cent Disease Incidence

The AgNPs was proved to be effective for the management nematode fungal disease complex involving *M. incognita* and *F. oxysporum* f.sp. *lycopersici* in tomato with cent per cent reduction in gall index and wilt incidence. Further, the treatment caused appreciable reduction in nematode population both in soil (58.43 %) and root

(64.29 %); number of females with egg mass/g root (80.00 %); eggs/ egg mass (24.86 %) (Table 29).

4.4.2.4.1.2. Plant growth parameters

The promising effect of AgNPs in the management of nematode fungal disease complex of tomato resulted in improvement in plant growth characters of shoot height (10.00 %) and weight (16.80 %); root length (35.00 %) and weight (37.85 %) compared to untreated control (Table 30).

4.4.2.4.2. Blackgram

4.4.2.4.2.1. Nematode population and Per cent Disease Incidence

As above the effectiveness of AgNPs was proved in the case of nematode fungal disease complex involving *M. incognita* and *M. phaseolina* with cent per cent reduction in nematode/ fungal disease incidence in blackgram. Besides the above trend of reduction in nematode population both in soil and root, number of females with egg masses and eggs/egg mass observed is furnished in Table 31.

4.4.2.4.2.2. Plant growth parameters

The suppression in nematode fungal disease complex due to application of AgNPs resulted in improvement in plant growth parameters *viz.*, shoot height (18.92 %) and weight (17.49 %); root length (3.84 %) and weight (31.79 %) compared to untreated control (Table 32).

4.4.2.5. Nematode bacterial disease complex

4.4.2.5.1. Brinjal

4.4.2.5.1.1. Nematode population and Per cent Disease Incidence

The results of the experiment conducted in plant growth chamber for the management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* in brinjal with AgNPs in comparison with untreated control are furnished in Tables 33 and 34.

The AgNPs had marked effect to check the nematode bacterial disease complex. It is interesting to note that there was absolutely no incidence of nematode and bacterial

wilt as measured by gall index and PDI in brinjal by the application of AgNPs at 45 DAT. Its effect reflected on nematode population, number of females with egg masses and fecundity of nematode with appreciable reduction and improvement in length and weight of shoot and root in brinjal.

4.5. Effect of green synthesized AgNPs on beneficial soil microorganisms

4.5.1. Entomopathogenic nematode *Steinernema glaseri*

The AgNPs caused cent per cent mortality of *S. glaseri* at higher concentrations of 0.0005 and 0.0001 per cent during different period of exposure of 1-5 days. Whereas the lower concentrations of 0.00005 and 0.00001 per cent of AgNPs caused reduced rate of mortality of *S. glaseri* initially and thereafter attained its highest level of 65.12 per cent on 5^{th} day of exposure. The effect of AgNPs causing adverse effect on *S. glaseri* is differing significantly among the different concentrations/period of exposure (Table 35 and Plate 34).

Therefore, it is concluded that the AgNPs was toxic to beneficial entomopathogenic nematode, *S. glaseri* being widely used for the management of insect pest management programme. However it needs further confirmation in future.

4.5.2. Fungal bioagents

4.5.2.1. *Trichoderma viride*

All the concentrations of AgNPs experimented in the present study were found to be inhibitory to *T. viride* being commonly used as bioagent for the management of plant pathogens including phytonematodes. Its harmful effects on test beneficial fungal biocontrol agent was positively correlated to the concentrations of the AgNPs (Table 36 and Plate 35).

Further studies on this line are warranted to confirm the illeffect of green synthesized AgNPs and to optimize the concentration of the same which is inhibitory only to target plant pathogen and not to other beneficial microorganisms.

4.5.2.2. *Paecilomyces lilacinus*

The above inhibitory effect of AgNPs was also observed with another beneficial fungus of *P. lilacinus* commonly known as nematode egg parasitic fungus. All the concentrations of 0.01 to 0.1 per cent of AgNPs caused significant inhibitory effect and it was less than 50 per cent over untreated control and its effect was found to be concentration dependent (Table 37 and Plate 36).

4.5.3. Bacterial bioagents

4.5.3.1. *Pseudomonas fluorescens*

The results of the experiment furnished in Table 38 showed that all the concentrations of AgNPs was proved to possess highest inhibitory effect to the bacterium with more than 90 per cent and it was positively correlated with the concentrations of AgNPs using *T. procumbens* in the present study. However, this preliminary observation needs confirmation in future (Plate 37).

4.5.3.2. *Bacillus subtilis*

As above the AgNPs was found to be inhibitory to the *B. subtilis* at different concentrations of 0.025 to 0.1 per cent (Plate 38). In this study, the AgNPs caused the highest inhibitory effect on the test bacterium at 0.1 per cent and its inhibitory effect was corresponded to the concentrations of the AgNPs (Table 38).

CHAPTER V

DISCUSSION

The findings of the present study on green synthesis and characterization of silver nanoparticles (AgNPs) using different plants and their antinemic effect on *Meloidogyne incognita*; antifungal effect on *Fusarium oxysporum* f.sp. *lycopersici* and *Macrophomina phaseolina*; antibacterial effect on *Ralstonia solanaceaerum* and *Xanthomonas oryzae* pv *oryzae in vitro*; under glasshouse conditions and in plant growth chamber besides their effects on beneficial entomopathogenic nematode, *Steinernema glaseri*; fungal bioagents *Trichoderma viride* and *Paecilomyces lilacinus*; bacterial bioagents *Pseudomonas fluorescens* and *Bacillus subtilis* are discussed in this chapter.

The nanotechnology permits broad advances in agricultural research for the management of plant diseases as well as to reduce the usage of synthetic chemical pesticides posing lot of illeffects to environment, nontarget organisms, consumer of agricultural produce etc., as stated by Patolsky *et al.* in the year 2006. Different types of physical and chemical methods are being employed for the synthesis of nanoparticles. However these methods were low in their production rate of nanoparticles. Further the required strong and weak chemical reducing agents and protective agents like sodium borohydride, sodium citrate and alcohols for these methods were reported to be toxic, flammable and cannot be dispersed easily off due to environmental issues as several authors reported by Mohanpuria *et al.* (2007); Rai *et al.* (2008); Sharma *et al.* (2009) and Bar *et al.* (2009).

Hence, Rai *et al.* (2009), Thakkar *et al.* (2010) and Margarita (2011) viewed that biological synthesis of AgNPs employing the usage of biological agents like bacteria, fungi, actinomycetes, yeast, algae and plants is desirable. In this method of biological synthesis of AgNPs, the use of plants is advantageous over other biological agents by eliminating the elaborate process of maintaining the cell culture. Further, the synthesis of nanoparticles using plants can be suitably scaled up and cost effective according to Parikh *et al.* (2005).

Therefore, an attempt was made for the green synthesis AgNPs using commonly available plants and to characterize the same. The results of the study is discussed critically as follows.

5.1. Green synthesis of AgNPs

The green synthesis of AgNPs was successful with the use of leaf extracts of *Tridax procumbens, Euphorbia hirta* and *Azardirachta indica* in the present study. Several authors achieved to green synthesis AgNPs using xerophytes, mesophytes and hydrophytes in general (Jha *et al.,* 2009) and particularly with the mesophytes like *Acalypha indica* (Krishnaraj *et al.,* 2010) *Trianthema decandra* (Geethalakshmi and Sarada, 2010) *Sesuvium portulacastrum* (Nabikhan *et al.,* 2010); *Ocimum sanctum* (Singhal *et al.,* 2011); *Garcinia mangostana* (Veerasamy *et al.,* 2011) *Nicotiana tobaccum* (Parasad *et al.,* 2011); *Ocimum tenuiflorum, Solanum trilobatum, Syzygium cumini, Centella asiatica* and *Citrus sinensis* (Logeswari *et al.,* 2015); *Ficus benghalensis* (Saxena *et al.,* 2012); and *Olea europaea* (Awwad *et al.,* 2012).

The findings of the present study fall in line with the report of successful synthesis of AgNPs with the plants of *T. procumbens* (Ondari and Nalini, 2014; Bhati and Malik, 2014 and Dhanalakshmi and Rajendran, 2012); *E. hirta* (Elumalai *et al*., 2010) and *A. indica* (Gavhane *et al.,* 2012 and Tripathy *et al.,* 2010). The environmentally benign and chemically complex nature of plants which contains biomolecules such as proteins, enzymes, amino acids, alkaloids, terpenoids, phenols, flavanoids, tannins, quinines etc., were reported to be responsible for the successful synthesis of AgNPs (Kavitha *et al,* 2013).

5.1.1. Characterization of AgNPs

The AgNPs size of 10 to 60 nm with spherical shape, stability, crystalline nature, higher content of silver elemental composition were considered as the desirable characters of green synthesized AgNPs (Shah *et al.,* 2009).

In the present study all the three plants *viz., T. procumbens, E. hirta* and *A. indica* used for the synthesis of AgNPs yielded the stable, crystalline particle size of 15 to 50 nm with spherical shape and higher silver elemental composition as reported by earlier workers (Krishnaraj *et al.,* 2010).

Among the three plants chosen for the synthesis of AgNPs the *T. procumbens* was considered as most preferable plant source owing to the following reasons.

5.1.1.1. Particle size

As per particle size analyzer the average particle size of AgNPs was within the limit of 100 nm (Ravikumar and Sathish Kumar, 2014) *viz., T. procumbens* (78.9 nm), *E. hirta* (87. 6 nm) and *A. indica* (40.4 nm) used for the green synthesis of AgNPs. Whereas the particle size of AgNPs using *T. procumbens* was found to be the least (15.5 to 31.8 nm) as per the TEM observations in the present study and it is coinciding with the report of Nagati *et al.* (2012) who stated that the size of synthesized AgNPs within 50 nm could be optimum size for silver nanoparticles.

The smallest size of nanoparticles having the highest surface volume ratio which enhance the surface energy is lethal to biological organisms as reported by Shrivastava *et al*. (2007). Therefore, the *T. procumbens* is highly preferred than other plant sources tried for the green synthesis of AgNPs.

5.1.1.2. Stability

It is reported that the stability of the green synthesized AgNPs is varying markedly (Kavitha *et al*., 2013). However, the high stability of nanoparticles was considered as one of the desirable characters of the synthesized AgNPs (Ondari and Nalini, 2014). The present study proved the stable nature of the synthesized AgNPs using all the three plants as measured in terms of Zeta potential and it was highest with *T. procumbens* (-41.7 mV). The finding is contradictory to the earlier report of low to moderate stability of the synthesized AgNPs using *T. procumbens* made by Ondari and Nalini (2014) and Kaveri (2014). However, the high stability of AgNPs synthesized using *T. procumbens* in the present study supported the preference of the same plant source for the synthesis of AgNPs.

5.1.1.3. Confirmation of formation of AgNPs

In UV-Vis spectra the absorbance peak at 435, 444 and 400 nm was observed due to change in colour from yellowish to brown on addition of $AgNO_3$ to plant extracts of *T. procumbens, E. hirta* and *A. indica* in the present study. Dhanalakshmi and Rajendran (2012); Elumalai *et al.* (2010) and Shankar *et al.* (2004) reported the

confirmation of formation of AgNPs could be made with absorbance peak ranging from 430 to 460 nm. The AgNPs synthesized in the present study using *T. procumbens* had the maxima absorbance of 435 nm which was within the range indicated.

Bhati and Malik (2014) and Ondari and Nalini (2014) explained that there was reduction of pure Ag^{+} to Ag^{0} ions due to splitting of $AgNO_3$ into Ag^{+} and NO_3^{-} during the synthesize of AgNPs. Hence change in colour in the reaction mixture was observed with progressive time. Apparently the metabolites in the leaf extract acted as e^{-} donor and reduced Ag^{+} ions into Ag.

5.1.1.4. Shape of AgNPs

The observations made through SEM revealed spherical shape of the AgNPs using all the above three plants with agglomeration. The observations made through TEM also confirmed the spherical shape of AgNPs. Dhanalakshmi and Rajendran (2012), Bhati and Malik (2014) and Ondari and Nalini (2014) also observed similar spherical shape of green synthesized AgNPs. Further, the spherical shape had already been documented in the literature (Elumalai *et al.* 2010).

5.1.1.5. Elemental compositions

The higher proportion of silver elemental composition in the green synthesized AgNPs is desirable (Paulkumar *et al.*, 2014; Ondari and Nalini, 2014; Gavhane *et al.*, 2012).

The TEM EDAX observations showed the specified peaks indicating the possession of silver in AgNPs using the above three plant sources (Ondari and Nalini, 2014 and Gavhane *et al.*, 2012). Further, the SEM EDAX analysis undertaken to determine the elemental composition also showed that there was highest silver elemental composition (97.91 %) in *T. procumbens* mediated synthesized AgNPs. The elemental composition of silver was lesser in AgNPs synthesized using certain plants (Khan *et al.*, 2013; Ravikumar and Sathish Kumar, 2014). Therefore, the higher elemental composition of silver as observed with *T. procumbens* mediated synthesized AgNPs in the present study made the plant as desirable source for the synthesis of AgNPs.

5.1.1.6. Crystalline nature

The sharp peak indicated by the X-ray diffraction pattern confirmed the crystalline nature of green synthesized AgNPs using all the above three plants in the present study and the findings confirmed the earlier report of Shankar *et al.* (2004); Sileikaite *et al*. (2009); Geoprincy *et al.* (2011) and Bhati and Malik (2014).

5.1.1.7. Plant extracts as reducing, capping and stabilizing agent

The FTIR analysis confirmed the presence of different functional group of phytochemicals in plants used for the synthesis of AgNPs. The phytochemicals were believed to act as reducing agent for the conversion of Ag^{+} to Ag^{0} and to act as capping and stabilizing agent of AgNPs in the present study. The findings support the earlier reports of Tripathy *et al.* (2010); Abduz Zahir *et al.* (2012); Ondari and Nalini (2014) and Bhati and Malik (2014).

It was speculated that the phytochemicals *viz.*, phenols, aldehydes, aromatic groups of the amino acid residues and proteins had strong ability to bind with metal for acting as reducing, capping and stabilizing agent of green synthesized AgNPs. Further, it is reported to be responsible for the prevention of agglomeration of green synthesized AgNPs. Therefore, it is concluded that the phytochemicals/biomolecules present in the above plant sources played a dual role in the formation and stabilization of the green synthesized AgNPs.

5.2. Evaluation of green synthesized AgNPs for their antimicrobial properties

Limited work carried out earlier on the effectiveness of metal silver nanoparticles and green synthesized AgNPs is encouraging (Pal *et al.*, 2007). Hence, the *T. procumbens* mediated green synthesized AgNPs possessing all the desirable traits of AgNPs was advanced to study for their antimicrobial properties and their adverse effects on beneficial organism as the work carried out on this line is very meagre.

5.2.1. Antinemic properties

The work carried out with chemically synthesized metal nanoparticles for their antinemic properties was more compared to the green synthesized AgNPs. The green synthesized AgNPs using *T. procumbens* in the present study prohibited the attraction of

J_2 of *M. incognita* in tomato. Further it is proved to be effective to inhibit the egg hatching (Fig. 17) and causing mortality of juveniles (Fig. 18) of the nematode with positive correlation between the concentrations of the green synthesized AgNPs/ time of exposure and its nematicidal effect (Figs. 19 and 20).

There was no reports on the antinemic properties of the green synthesized AgNPs. However the results of the study conducted by Ardakani (2013) and Cromwell *et al.,* (2014) to evaluate the antinemic properties of metal AgNPs against *M. incognita* and *M. graminicola* support the present findings. The present results of effectiveness of AgNPs against *M. incognita* seems to be a first report. Therefore it is opined that the work on this line needs to be intensified to confirm the results of the present study.

The metal nanoparticles responsible for induction of oxidative stress in the cells of phytonematodes and their direct toxicity over the metabolic activities of nematodes were explained as possible mechanism of AgNPs by Yeon Roh *et al.* (2009) and Lim *et al.* (2012).

5.2.2. Antifungal properties

The present findings proved the effectiveness of the AgNPs to inhibit the growth and development of mycelium and per cent disease incidence of *F. oxysporum* f.sp. *lycopersici* (Fig. 21) and *M. phaseolina* (Fig. 22). The antifungal activity of the AgNPs was found to be directly proportional to its concentrations/ time of exposure.

No studies have been made on the antifungal property of AgNPs following the method of green synthesis using different plants. However, informations available on the promising results of chemically synthesized AgNPs against various fungal pathogens of *Altereria alternate, Sclerotinia sclerootiorum, M. Phaseolina, Rhizoctonia solani, Botrytis cinerea, Curvuleria lunata, Aspergillus niger, A. flavus, F. oxysporum, F. moniliforme* and *Fusarium* spp. support the present findings (Krishnaraj *et al.,* 2012; Linga Rao and Savithramma, 2012 and Bholay *et al.,* 2013).

In an attempt of studying the mechanism of chemical and green synthesized AgNPs by Morones *et al.* (2005) it is reported that the AgNPs disturb transport systems including ion efflux. The dysfunction of ion efflux causing rapid accumulation of silver

ions and interrupt cellular processes such as metabolism and respiration of target organisms. Further, it is speculated that the AgNPs penetrate the cell wall, affect the DNA and its ability to inactivate expression of ribosomal subunit proteins as well as certain cellular proteins and enzymes essential for ATP production (Shrivastava *et al.*, 2007). Besides, AgNPs were found to be responsible to inactivate microbial enzymes and thereby facilitating production of reactive oxygen species (ROS) which is detrimental to cells of plant pathogenic fungi and to cause damage to proteins, lipids and nucleic acids (Storz and Imlay, 1999 and Hwang *et al.,* 2008).

Therefore in continuation of above studies Allahverdiyev *et al.* (2011) proving AgNPs leads to cell death of microbes support the present findings.

5.2.3. Antibacterial properties

The green synthesized AgNPs proved to be antibacterial activities against *R. solanacearum* (Fig. 23) and *X. oryzae* pv *oryzae* (Fig. 24) and its effect was found to be concentration dependent. The AgNPs synthesized using plant sources *viz.,* oak (*Quercus infectoria*) and delile (*Ulva fasciata*) was effective against many bacterial pathogens including *R. solanacearum* and *X. oryzae* pv *oryzae* support the present findings (Park *et al.,* 2006, Sahayaraj *et al*., 2012 and Mahmood Chahardooli *et al*., 2014).

Compared to nematode and fungal pathogens the general mechanisms of AgNPs against bacterial pathogens is well documented as detailed below and the same is explained as possible mechanism of *T. procumbens* mediated green synthesized AgNPs in the suppression of *R. solanacearum* and *X. oryzae* pv *oryzae*.

The death of bacteria due to loss of cell permeability was reported when the positively charged AgNPs accumulate on negatively charged cell membrane of bacterial pathogens and the same is explained as possible mechanism of chemical/green synthesized AgNPs by Ramamurthy *et al.* (2013), Hamouda and Baker (2000) and Mubarak Ali *et al.* (2011). In addition Sondi and Salopek Sondi (2004); Morones *et al.* (2005); Mahendra *et al.* (2009) observed that AgNPs entering the bacterial cell and releasing the silver ions, attack the respiratory chain and interfere with the bacterial growth signaling pathway by modulating tyrosine phosphorylation of putative peptides substrates which are essential for cell viability and division.

It is also reported that bacterial cell membrane permeability was affected by progressive release of lipopolysaccharides and membrane proteins when treated with AgNPs and the same is responsible for the formation of irregularly shaped pits in the outer membrane of the bacteria. Besides the AgNPs interact with the sulfur containing proteins of the bacterial cell membrane and denaturing them. In the same way the interaction of AgNPs with the phosphorus containing compounds cause damage to DNA with resultant death of bacterial cells (Amro *et al.,* 2000 and Guzman *et al.,* 2012).

5.2.4. Morphological changes due to green synthesized AgNPs.

5.2.4.1. Nematodes

In this preliminary study the egg mass of *M. incognita* on exposure to green synthesized AgNPs were found to be malformed. The juveniles of the root knot nematode appeared to be deformed with oozing out of its body fluid. The reason for the deformity of nematode caused by the green synthesized AgNPs is not known and absolutely no work has been carried out on this line. Hence further studies to explore the possibility of green synthesized AgNPs for causing morphological changes in the nematodes is warranted.

5.2.4.2. Fungi

In the present study, the green synthesized AgNPs caused crinkling and shriveling besides resulting in uneven surface of the hyphae of the test fungi *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina*. The present results of malformation and deformity of the fungi is in accordance with the report of Kim *et al.* (2008) and Hassan *et al.* (2013) who reported that the green synthesized AgNPs were capable of damaging and collapsing the cell wall; deforming hyphae; breaking mycelia; tearing hyphal wall and causing swollen margins of the hyphae as malformation and thereby reducing the ability of the pathogen to regenerate, multiply and cause diseases.

5.3. Management of root knot nematode, fungi, bacteria and their disease complex under glasshouse conditions

5.3.1. Root knot nematode

Information available on the management of phytonematodes using chemical/green synthesized AgNPs in comparison with other recommended practices is

inadequate. Therefore, the green synthesized AgNPs using the most preferred plant source of *T. procumbens* with the concentration 0.0005 per cent based on the results of laboratory study was evaluated against the root knot nematode *M. incognita* in comparison with the standard practices of use of biocontrol agent *Pseudomonas fluorescens* and chemical nematicide carbofuran 3G on tomato.

From the Figures 25 and 26 it is apparent that the green synthesized AgNPs was more effective than *P. fluorescens* and carbofuran 3G for the management of *M. incognita* in tomato and to increase the yield attributes of the crop.

The green synthesized AgNPs had highest effect in checking *M. incognita* population in soil (88.16%), root (92.79), number of females with egg masses (79.80), eggs/egg mass (65.14%) and nematode disease severity in terms of gall index. The significant negative effect of AgNPs on *M. incognita* reflected on tomato plant growth with increased shoot height (26.99%) and weight (13.92%); root length (26.57%) and weight (23.45%) and fruit yield (20.54%).

The earlier report of Ardakani (2013) on the toxicity of different nanoparticles *viz.,* AgNPs, SiO_2NPs and TiO_2NPs against *M. incognita* support the present findings. However, the author reported that the metal nanoparticles were toxic to nematodes as well as the crop tomato. Therefore the later part of the findings of the author is contradictory to the present findings. However, the recent finding of Cromwell *et al.* (2014) indicated that the AgNPs were nematicidal to *M. graminis* and improved the growth and quality of turfgrass coincided with the present findings.

Wang *et al.* (2009) experimented on the nematicidal activity of Al_2O_3NPs, ZnONPs and TiO_2NPs against *Caenorhabditis elegans* for the first time and proved that all the tested metal nanoparticles affect the survival, growth, length of nematode body and fecundity of nematodes.

Subsequent informations available on the mechanism of AgNPs indicated that the AgNPs inhibit the survival, growth and reproduction of nematodes. The increased expression of superoxide dismutase and abnormal dauer formation protein genes were considered as reason for the mechanism of AgNPs by Yeon Roh *et al.* (2010). In continuation Lim *et al.* (2012) hypothesized that induction of oxidative stress in the

cells and their direct toxicity on metabolic activities of nematodes as an important mechanism of AgNPs in the suppression of the population of free living nematode *C. elegans*.

Therefore, the present preliminary study clearly indicated that the green synthesized AgNPs was more effective for the management of *M. incognita* on tomato. In this regard the author opined a long term plan is required in future to confirm the effectiveness of AgNPs under field conditions after fulfilling the rules and regulations for the same.

5.3.2. Fungal plant pathogens

Considerable work has been carried out on the antifungal activity of AgNPs *in vitro* but it was negligible under controlled glasshouse conditions.

Therefore attempts were made in the present study to confirm the *in vitro* antifungal effect of the green synthesized AgNPs in pot experiments under controlled glasshouse conditions for the management of *F. oxysporum* f.sp. *lycopersici* (Fig. 27) and *M. phaseolina* (Fig. 28) in tomato and blackgram respectively. The experimental results revealed that the green synthesized AgNPs at 0.1 per cent was highly effective than biocontrol agent and chemical fungicide to bring down the PDI caused by *F. oxysporum* f.sp. *lycopersici* (60.52 %) and *M. phaseolina* (50.26 %) and to improve the plant growth characters and ultimately economic yield by 14.77 and 56.71 per cent in tomato and blackgram respectively. So far no pot culture experiments were conducted for the antifungal activity of green synthesized AgNPs against both fungi tested in the present study.

However, the literature documented already on the effectiveness of AgNPs against *Sclerotium cepivorum*, *Magnaporthe grisea* and *Bipolaris sorokiniana* in onion, rice and wheat under greenhouse conditions support the present findings (Park *et al.*, 2006). Further the present finding was confirmed by the recent report of Lamsal *et al.* (2011) who stated that the high fungal inhibitory effects of AgNPs resulted in increase in plant biomass with dose dependent manner.

5.3.3. Bacterial plant pathogens

The present findings of higher effectiveness of the green synthesized AgNPs at 0.1 per cent compared to biocontrol potential of *P. fluorescens* and streptomycin sulphate used as chemical in the management of bacterial wilt disease caused by *R. solanacearum* in tomato seems to be a first report. The suppression of bacterial wilt disease incidence (56.01 %) by the green synthesized AgNPs had resulted in increased plant growth characters including fruit yield by 16.96 per cent (Fig. 29).

Related study conducted by Ismail Ocsoy *et al*. (2013) proving the antibacterial effect of AgNPs in the management of bacterial spot disease caused by *Xanthomonas perforansa* in tomato under controlled conditions support the present findings.

5.3.4. Nematode fungal disease complex

Experiments conducted under controlled glasshouse conditions for the effect of green synthesized AgNPs against nematode fungal disease complex involving *M. incognita* with *F. oxysporum* f.sp. *lycopersici* in tomato (Figs. 30 and 31) and *M. incognita* with *M. phaseolina* in blackgram (Figs. 32 and 33) revealed the profound effect of green synthesized AgNPs in the management of nematode fungal disease complex and to improve biomass of the test crops. Since absolutely no work has been carried out on this line elsewhere the present findings could not be matched.

Nevertheless the significant inhibitory effect of the green synthesized AgNPs individually against *M. incognita / F. oxysporum* f.sp. *lycopersici/ M. phaseolina* as observed in the present study is related for the simultaneous antinemic cum antifungal effect of the green synthesized AgNPs in the management of nematode fungal disease complex of tomato and blackgram.

5.3.5. Nematode bacterial disease complex

For the first time it is reported that the green synthesized AgNPs using *T. procumbens* at 0.1 per cent had effect for the management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* with subsequent improvement in yield attributes of brinjal under controlled glasshouse conditions (Figs. 34 and 35). No literature is available to compare the findings of the present study.

Therefore significant effect of the green synthesized AgNPs individually against test nematode and bacterium as proved in the present study *in vitro* is considered as reason for the effectiveness of green synthesized AgNPs in the management of nematode bacterial disease complex of brinjal.

5.4. Reconfirmation of effectiveness of the green synthesized AgNPs

In continuation of confirming the antinemic/ antifungal/ antibacterial activities of the green synthesized AgNPs *in vitro* and under controlled glasshouse conditions on different crops it is desired to reconfirm the same in plant growth chamber.

5.4.1. Against root knot nematode

The preliminary study initiated on this line in plant growth chamber clearly reconfirmed the possession of antinemic property by the AgNPs green synthesized using *T. procumbens* in the present study.

The AgNPs registered considerable reduction in *M. incognita* population in soil, root, number of females with egg masses and eggs/egg mass and caused marked improvement in plant growth of tomato over untreated control. Therefore the effectiveness of AgNPs was reconfirmed but could not be compared with earlier reports as no attempt has been made to study the antinemic property of chemical/ green synthesized AgNPs in plant growth chamber.

5.4.2. Against fungi

The *T. procumbens* mediated green synthesized AgNPs caused complete suppression of disease incidence caused by *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* respectively at early stage of tomato and blackgram and improved the growth of the plants. Therefore, the present experimental results of antifungal effect of AgNPs *in vitro* and controlled glasshouse conditions was reconfirmed in plant growth chamber also.

Jo and Kim (2009) observed that the AgNPs directly contacting spores and germ tube of *B. sorokiniana* and *M. grisea* inhibited their viability and influenced spore colonization and disease expression *in vitro* and proved the same on perennial ryegrass in plant growth chamber and concluded that the AgNPs could be used as preventive

measure for the management of the above fungal disease in ryegrass. Therefore the reconfirmation of antifungal effect of AgNPs in the present study coincided with the report of Jo and co-workers in 2009.

5.4.3. Against bacteria

The antibacterial effect of green synthesized AgNPs was reconfirmed in plant growth chamber through the complete suppression of wilt incidence caused by *R. solanacearum* on brinjal with improvement in plant biomass over untreated control. As mentioned above no suitable literature is available to compare the present findings.

5.4.4. Against nematode fungal disease complex

The work carried out for the management of either nematode fungal or nematode bacterial disease complex using AgNPs is absolutely nil. Therefore the work on this line was initiated under different conditions including plant growth chamber.

In plant growth chamber the effectiveness of green synthesized AgNPs at 0.1 per cent was reconfirmed for the management of root knot nematode fungal disease complex with *F. oxysporum* f.sp. *lycopersici* in tomato and *M. phaseolina* in blackgram and to improve the plant growth characters. Although the antinemic (Ardakani, 2013) or antibacterial (Mahmood Chahardooli *et al.*, 2014) effects of the AgNPs was established individually *in vitro* and pot conditions no such studies have been carried out for the management of nematode fungal disease complex that too in plant growth chamber. Hence the present finding having literature value needs to be confirmed in future.

5.4.5. Nematode bacterial disease complex

The green synthesized AgNPs experimented on brinjal suppressed the nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* in plant growth chamber and caused marked improvement in plant growth characters compared to untreated control. No literature is available to support the present findings. Hence it is opined that future line of work is required to confirm the effectiveness of AgNPs for the management of nematode bacterial disease complex.

5.5. Effect of green synthesized AgNPs on beneficial organisms

5.5.1. Against entomopathogenic nematode

The green synthesized AgNPs was found to be lethal to the entomopathogenic nematode, *S. glaseri* and its adverse effect was found to increase with increase in the concentrations of AgNPs from 0.00001 to 0.0005 per cent and time of exposure (Fig. 36).

The earlier work of Kucharska *et al.* (2009 and 2014) reporting adverse effect of AgNPs with 0.5 and 5 ppm concentrations and five days period of exposure on *S. feltiae* and *Heterorhabditis bacteriophora* strengthen the present findings.

The majority of the reports support the lethal effect of chemical or green synthesized AgNPs against nematode, fungal and bacterial pathogens indicating the broad spectrum of action (Sondi and Salopek Sondi, 2004; Morones *et al.,* 2005; Lok *et al.,* 2007; Choi *et al.,* 2008; Yeon Roh *et al.,* 2009; Ahamed *et al.,* 2011 and Lim *et al.,* 2012). Therefore it is considered that the intensification of the work on this line to optimize the dosage of AgNPs which is harmful to plant pathogens but not to beneficial organisms is a challengeable task to the scientists working in this field.

5.5.2. Against fungal bioagents

All the concentrations of 0.01 to 0.1 per cent of the green synthesized AgNPs inhibited the growth and development of commercially available beneficial fungi of *T. viride* and *P. lilacinus* being widely used for the management of plant diseases (Fig. 37).

Earlier report of inhibitory effect of AgNPs on *T. viride* and *T. harzianum* which is depending on concentrations and period of exposure support the present findings (Gavanji *et al*., 2012). Therefore it is suggested to fix the optimum concentration of AgNPs which is lethal to plant pathogens but not to beneficial fungi.

5.5.3. Against bacterial bioagents

All the concentrations of 0.001 to 0.1 per cent of the green synthesized AgNPs were found to be harmful to the disease causing bacterial pathogens of *R. solanacearum* and *X. oryzae* pv. *oryzae* as mentioned earlier and also beneficial bacteria of *P. fluorescens* and *B. subtilis*. However the higher concentrations upto 0.1 per cent proved to be bactericidal irrespective of plant disease causing or beneficial bacteria

experimented in the present study (Fig. 38). Hence the present findings leads to the credence of Park *et al.* (2006) who proved that the AgNPs caused cent per cent growth inhibition on beneficial bacteria like *B. subtilis* and *Azotobacter chroococum.*

The studies clearly indicated that AgNPs synthesized in the present study had broad spectrum of action against nematodes, fungal and bacterial pathogens. However, the AgNPs was also having antinemic, antifungal and antibacterial activity against *S. galseri, T. viride, P. lilacinus, P. fluorescens* and *B. subtilis*. This indicate that, to develop the biocontrol agents resistant to AgNPs which will pave the way for IPM.

CHAPTER VI

SUMMARY

The salient findings of the present study on green synthesis of AgNPs using different plants *viz., Tridax procumbens*, *Euphorbia hirta* and *Azardirachta indica* and their characterization in addition to their antinemic, antifungal and antibacterial effects under laboratory, glasshouse conditions and plant growth chamber; effect on morphology of plant pathogens; harmful effects on beneficial entomopathogenic nematode; fungal and bacterial bioagents are summarized below.

1. The green synthesis of AgNPs was successful with all the three plants *viz., T. procumbens*, *E. hirta* and *A. indica* evaluated.
2. The green synthesized AgNPs characterized for their size, shape, stability, crystalline nature, Ag elemental composition and ability of plant biomolecules to act as reducing, capping and stabilizing agent of AgNPs were compared to identify the most desirable plant sources.
3. Accordingly the *T. procumbens* confirming the synthesis of AgNPs with appropriate absorbance peak of 435 nm was rated as most desirable plant for the green synthesis of AgNPs compared to other two plants.
4. The *T. procumbens* mediated green synthesized AgNPs were having the spherical shape of particles which are highly stable and crystal in their nature. Further, the particle size was also lowest from 15.5 to 31.8 nm with highest Ag elemental composition by 97.91 per cent.
5. Besides the *T. procumbens* was found to be highly suitable than *E. hirta* and *A. indica* for acting as reducing agent as well as capping and stabilizing agent of AgNPs in the attempt of green synthesizing AgNPs.
6. *In vitro* the *T. procumbens* mediated green synthesized AgNPs were found to influence the nematode behavior of attraction and penetration of *M. incognita* towards root of tomato. Similarly the AgNPs were proved to be ovicidal and

larvicidal to root knot nematode. In this regard the effect of AgNPs was found to be positively correlated with their concentrations and time of exposure.

7. As above the green synthesized AgNPs using *T. procumbens* proved to inhibit the growth and development of fungal pathogens *viz.*, *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* and its effect was directly proportional to their concentrations and time of exposure *in vitro*.

8. The green synthesized AgNPs utilizing the plant source of *T. procumbens* were also found to be bactericidal *in vitro* as the AgNPs showed the highest zone of inhibition with *R. solanacearum* and *X. oryzae* pv *oryzae*. The bactericidal effect of AgNPs depends on the concentrations of AgNPs.

9. No change was induced over eggs of root knot nematode by the green synthesized AgNPs while the bodily structure of *M. incognita* juveniles were found to be malformed with oozing out of its body fluid. In the same way the mycelium/hyphae of *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* were found to be discolored, discontinued, distorted, crinkled and shriveled on exposure to the AgNPs.

10. The *in vitro* antinemic effect of green synthesized AgNPs was also confirmed under glasshouse conditions. The tomato plants treated with the AgNPs registered the lowest root knot nematode population/ incidence accompanied with increase in plant biomass.

11. As above the antifungal effect of green synthesized AgNPs was established under glasshouse conditions with the fungal pathogens of *F. oxysporum* f.sp. *lycopersici* in tomato and *M. phaseolina* in blackgram. In this study the plants treated with AgNPs recorded the lowest per cent disease incidence coupled with highest fruit/seed yield.

12. The green synthesized AgNPs were also found to possess antibacterial property besides antinemic and antifungal effect and it is proved with the suppressive effect of AgNPs over wilt causing bacterial pathogen of *R. solanacearum* with resultant subsequent increase in plant biomass of brinjal.

13. The antinemic/ antifungal/ antibacterial effect of green synthesized AgNPs at 0.1 per cent was found to be in higher side when compared to *P. fluorescens* and synthetic chemical pesticides under glasshouse conditions.

14. The above trend was observed in the management of root knot nematode fungal disease complex involving with *F. oxysporum* f.sp. *lycopersici* and *M. phaseolina* in tomato and blackgram respectively and root knot nematode bacterial disease complex involving with *R. solanacearum* in brinjal under glasshouse conditions.

15. The profound effect of green synthesized AgNPs using *T. procumbens* was reconfirmed in plant growth chamber against *M. incognita*, *F. oxysporum* f.sp. *lycopersici* and their disease complex in tomato.

16. Similarly the effect of AgNPs against *M. phaseolina*, nematode fungal disease complex involving *M. incognita* and *M. phaseolina* in black gram was proved in plant growth chamber.

17. The above trend was also exhibited by the AgNPs against bacterial plant pathogen of *R. solanacearum* and nematode bacterial disease complex caused by *M. incognita* in association with *R. solanacearum* on brinjal.

18. The effective management of nematode, fungal/bacterial plant pathogens and their disease complex in different test crops using AgNPs resulted in marked improvement in plant growth upto 45 days after transplanting.

19. In general the green synthesized AgNPs using *T. procumbens* were found to be detrimental to all the tested beneficial organisms *viz.,* entomopathogenic nematode, *S. glaseri;* fungal biocontrol agents of *Trichoderma viride* and *Paecilomyces lilacinus* and bacterial biocontrol agents of *Pseudomonas fluorescens* and *Bacillus subtillis.*

REFERENCES

Abasaheb, R., C. Nalwade and A.A. Jadhav. 2013. Biosynthesis of silver nanoparticles using leaf extract of *Datura alba Nees.* and evaluation of their antibacterial activity. **Arch. Appl. Sci. Res., 5 (3):** 45-49.

Abduz Zahir, A., A. Bagavan, C. Kamaraj, G. Elango and A. Abdul Rahuman. 2012. Efficacy of plant mediated synthesized silver nanoparticles against *Sitrophilus oryzae*. **Journal of Biopesticides, 5**: 95 – 102.

Ahamed, M., M.A. Khan, M.K.J. Siddiqui, M.S. Alsalhi and S.A. Alrokayan. 2011. Green synthesis, characterization and evaluation of biocompatibility of silver nanoparticles. **Physica. E., 43**: 1266–1271.

Ahmad, A., P. Mukherjee, S. Senapati, D. Mandal, M.I. Khan, R. Kumar and M. Sastry. 2003. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. **Colloids Surf. B: Biointerfaces, 28**: 313-318.

Ahmad, N and S. Sharma. 2012. Green synthesis of silver nanoparticles using extracts of *Ananas comosus.* **Green Sustainable Chem., 2:** 141-147.

Ahmad, N., S. Sharma, M.K. Alam, V.N. Singh, S.F. Shamsi, B.R. Mehta and A. Fatma. 2010. Rapid synthesis of silver nanoparticles using dried medicinal plant of basil. **Colloids Surf. B: Biointerfaces, 81**: 81-86.

Ahmad, N., S. Sharma, V.N. Singh, S.F. Shamsi, A. Fatma and B.R. Mehta. 2011. Biosynthesis of silver nanoparticles from *Desmodium triflorum*: A novel approach towards weed utilization. **Biotechnol. Res. Int., 6:** 1-3.

Ahmad, Z., R. Pandey, S. Sharma and G.K. Khuller. 2006. Alginate nanoparticles as antituberculosis drug carriers: Formulation development, pharmacokinetics and therapeutic potential. **Indian J. Chest Dis. Allied Sci., 48**: 171-176.

Ali, D.M., N. Thajuddin, K. Jeganathan and M. Gunasekaran. 2011. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. **Colloids Surf. B: Biointerfaces, 85**: 360- 365.

Allah Ditta. 2012. How helpful is nanotechnology in agriculture -Advances in natural sciences: nanoscience and nanotechnology. **Adv. Nat. Sci.: Nanosci. Nanotechnol., 3**: 1-10.

Allahverdiyev, A.M., E.S. Abamor, M. Bagirova, C.B. Ustundag, C. Kaya and M. Rafailovich. 2011. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultra violet. **Int. J. Nanomedicine., 6**: 2705–2714.

Amarendra, D.D and G. Krishna. 2010. Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. **Colloid Surface, 369(3)**: 27-33.

Amin, M., F. Anwar, M.R.S.A. Janjua, M.A. Iqbal and U. Rashid. 2012. Green synthesis of silver nanoparticles through reduction with *Solanum xanthocarpum* L. berry extract: Characterization, antimicrobial and urease inhibitory activities against *Helicobacter pylori*. **Int. J. Mol Sci., 13**: 9923- 9941.

Amro, N.A., L.P. Kotra, K. Wadu Mesthrige, A. Bulychev, S. Mobashery and G. Liu. 2000. High-resolution atomic force microscopy studies of the *Escherichia coli* outer membrane: structural basis for permeability. **Langmuir, 16**: 2789- 2796.

Anil Kumar, S., M.K. Abyaneh, S.W. Gosavi Sulabha, A. Ahmad and M.I. Khan. 2007. Nitrate reductase mediated synthesis of silver nanoparticles from $AgNO_3$. **Biotechnol. Lett., 29**: 439–445.

Ankamwar, B. 2010. Biosynthesis of gold nanoparticles (Green gold) using leaf extract of *Terminalia catappa*. **European Journal of Organic Chemistry, 7**: 1334-1339.

Antonya, J.J., P. Sivalingam, D. Siva, S. Kamalakkannan, K. Anbarasu, R. Sukirtha, M. Krishnan and S. Achiraman. 2011. Comparative evaluation of antibacterial activity of silver nanoparticles synthesized using *Rhizophora apiculata* and glucose. **Colloids and Surfaces B: Biointerfaces, 88**: 134-140.

Ardakani, A.S. 2013. Toxicity of silver, titanium and silicon nanoparticles on the root knot nematode, *Meloidogyne incognita* and growth parameters of tomato. **Nematology, 15**: 671-677.

Arun kumar, C., A. Astalakshmi, P. Nima and V. Ganesan. 2013. Plant mediated synthesis of silver nanoparticles using leaves of *Odina wodier* Roxb**. Int. J. Adv. Res., 1(5):** 265-272.

Ashok Kumar, D. 2012. Rapid and green synthesis of silver nanoparticles using the leaf extracts of *Parthenium hysterophorus*: A novel biological approach. **International Research Journal of Pharmacy, 3**: 169-170.

Awwad, A.M., N.M. Salem and A.O. Abdeen. 2012. Biosynthesis of silver nanoparticles using *Olea europaea* leaves extract and its antibacterial activity. **Nanosci. Nanotechnol., 2(6):** 164-170.

Azmi, M.I and M. S. Jairajpuri. 1976. Mechanism of locomotion in *Hoplolaimus indicus* and *Helicotylenchus indicus*. **Nematologica, 22**: 277-283.

Baker, C., A. Pradhan, L. Pakstis, D.J. Pochan and S.I. Shah. 2005. Synthesis and antibacterial properties of silver nanoparticles. **J. Nanosci. Nanotechnol., 9(2)**: 244– 252.

Bakshi, M., H.B. Singh and P.C. Abhilash. 2014. The unseen impact of nanoparticles: More or less. **Curr. Sci., 106**: 350–352.

Banadkouki, H.Z., R.T. Afshari, J. Shaterian and H. Michael. 2013. Do nano silver treatments control dry rot agent inoculated young and aged potato seed tubers?. **International Journal of Agronomy and Plant Production, 4(12)**: 3301-3307.

Bar, H., D.K. Bhui, G.P. Sahoo, P. Sarkar, S.P. De and A. Misra. 2009. Green synthesis of silver nanoparticles using latex of *Jatropha curcas*. **Colloids Surf A: Physicochem. Eng. Asp., 339**: 134–139.

Barrena, R., E. Casals, J. Colon, X. Font, A. Sanchez and V. Puntes. 2009. Evaluation of the ecotoxicity of model nanoparticles. **Chemosphere, 75**: 850–857.

Basavaraja, S., S.D. Balaji, A. Lagashetty, A.H. Rajasab and A. Venkataraman. 2008. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. **Material Research Bulletin, 43**: 1164-1170.

Beesley, J.E. 1989. **Colloidal Gold: Principles, Methods and Applications**, Ed. M.A Hayat, Academic Press, New York, USA. Pp: 421-425.

Bergeson, L.L. 2010. Nanosilver pesticide products: What does the future hold?. **Environ. Qual. Manage, 19**: 73-79.

Bhat, R., S. Ganachari, P. Raghunandan, R. Deshpande, G. Ravindra and A. Venkataraman. 2013. Rapid biosynthesis of silver nanoparticles using Arecanut (*Areca catechu*) extract under microwave-assistance. **Journal of Cluster Science, 24:**107–114.

Bhati, H and C.P. Malik. 2014. Biosynthesis of silver nanoparticles using fresh extracts of *Tridax procumbens* Linn. **Indian Journal of Experimental Biology, 52:** 359-368.

Bholay, A.D., P.M. Nalawade and B.V. Borkhataria. 2013. Fungicidal potential of biosynthesized silver nanoparticles against phytopathogens and potentiation of fluconazole. **World Research Journal of Pharmaceutical Research, 1(1):** 12-15.

Bindhu, M.R and M. Umadevi. 2013. Synthesis of monodispersed silver nanoparticles using *Hibiscus cannabinus* leaf extract and its antimicrobial activity. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 101**: 184-190.

Binupriya, A.R., M. Sathishkumar and S.I. Yun. 2010. Mycocrystallization of silver ions to nanosized particles by live and dead cell filtrates of *Aspergillus oryzae* var. *viridis* and its bactericidal activity towards *Staphylococcus aureus* KCCM 12256. **Ind. Eng. Chem. Res., 49**: 852-858.

Bordes, P., E. Pollet and L. Averous. 2009. Nanobiocomposites: Biodegradable polyester! nanoclay systems. **Progr. Polym. Sci., 34**: 125-155.

Boruah, S.K., P.K. Boruah, P. Sarma, C. Medhi and O.K. Medhi. 2012. Green synthesis of gold nanoparticles using *Camellia sinensis* and kinetics of the reaction. **Adv. Mat. Lett., 3**: 481- 486.

Bouwmeester, H., S. Dekkers, M.Y. Noordam, W.I. Hagens and A.S. Bulder. 2009. Review of health safety aspects of nanotechnologies in food production. **Regul. Toxicol. Pharmacol., 53**: 52-62.

Carmen, I.M., P. P. Chithra, Q. Huang, T. Paul, S. Liu and L. Kokini Jozef. 2003. Nanotechnology: A new frontier in food Science. **Food Technology, 57**: 24-29.

Chittaranjan, K, K. Phullara, R. Manoj, P. Choudhary, P. Ramakrishna, A. Rao and R. Richard. 2013. Nanobiotechnology can boost crop production and quality: First evidence from increased plant biomass, fruit yield and phytomedicine content in bittermelon (*Momordica charantia*). **BMC Biotechnology, 13(37)**: 1-10.

Choi, O., K.K. Deng, N.J. Kim, L. Ross, R.Y. Surampalli and Z. Hu. 2008. The inhibitory effects of silver nanoparticles, silver ions and silver chloride colloids on microbial growth. **Water Res., 42**: 3066–3074.

Chowdappa, P., C. Shivakumar Gowda, S. Chethana and S. Madhura. 2014. Antifungal activity of chitosan silver nanoparticle composite against *Colletotrichum gloeosporioides* associated with mango anthracnose. **African Journal of Microbiology Research, 8(17)**: 1803-1812.

Christensen, L., S. Vivekanandhan, M. Misra1 and A.K. Mohanty. 2011. Biosynthesis of silver nanoparticles using *Murraya koenigii* (curry leaf): An investigation on the effect of broth concentration in reduction mechanism and particle size. **Advanced Materials Letters, 2**: 429-434.

Cioffi, N., L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli and L. Sabbatini. 2005. Copper nanoparticles/polymer composites with antifungal and bacteriostatic properties. **Chem. Mater., 17**: 5255-5262.

Cristobal, L.F., G.A. Castanon, R.E. Martinez, J.P. Rodriguez, N. Patino Marin, J.F. Reyes-Macias and F. Ruiz. 2009. Antibacterial effect of silver nanoparticles against *Streptococcus mutans*. **Mater. Lett., 63**: 2603–2606.

Cromwell, W.A., J. Yang, J.L. Starr and Young Ki Jo. 2014. Nematicidal effects of silver nanoparticles on root knot nematode in bermuda grass. **Journal of Nematology, 46(3)**: 261–266.

Dameron, C.T., R.N. Reese, R.K. Mehra, A.R. Kortan and P.J. Carroll. 1989. Biosynthesis of cadmium sulphide quantum semiconductor crystallites. **Nature, 338**: 596-597.

Daniel, S.C., S. Ayyappan, N.J.P. Philiphan, M. Sivakumar, G. Menaga and T.A. Sironmani. 2011. Green synthesis and transfer of silver nanoparticles in a food chain through chiranamous larva to zebra fish - A new approach for therapeutics. **Int. J. NanoSci. Nanotech., 2**: 159-169.

Danilczuk, M., A. Lund, J. Saldo, H. Yamada and J. Michalik. 2006. Conduction electron spin resonance of small silver particles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 63**: 189 - 191.

Deshpande, R., D.M. Bedre, S. Basavaraja, B. Sawle, S.Y. Manjunath and A. Venkataraman. 2010. Rapid biosynthesis of irregular shaped gold nanoparticles from macerated aqueous extracellular dried clove buds (*Syzygium aromaticum*) solution. **Colloids Surf B Biointerfaces, 79**: 235- 240.

Dhanalakshmi, R and S. Rajendran. 2012. Synthesis of silver nanoparticles using *Tridax procumbens* and its antimicrobial activity. **Arch. Applied Sci. Res., 4**: 1289-1293.

Dinesh, S., S. Karthikeyan and P. Arumugam. 2012. Biosynthesis of silver nanoparticles from *Glycyrrhiza glabra* root extract. **Arch. Appl. Sci. Res., 4**: 178-187.

Dipankar, C and S. Murugan. 2012. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. **Colloids and Surfaces B: Biointerfaces, 98**: 112– 119.

Duran, N., P.D. Marcato, G.I.H. De Souza, O.L. Alves and E. Esposito. 2007. Antibacterial effect of silver nanoparticles produced by fungal process on textile fabrics and their effluent treatment. **J. Biomed. Nanotechnol., 3**: 203-208.

Duran, N., P.D. Marcato, O.L. Alves, G.I.H. De Souza and E. Esposito. 2005. Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains. **J. Nanobiotechnol., 3**: 8–14.

Dwivedi, A.D and K. Gopal. 2011. Plant mediated biosynthesis of silver and gold nanoparticles. **J. Biomed Nanotechnol., 7**: 163- 164.

Elamawi, B.C, M.A. Rabab and R.A.S. El-Shafey. 2013. Inhibition effects of silver nanoparticles against rice blast disease caused by *Magnaporthe grisea*. **Egypt. J. Agric. Res., 91 (4)**: 1271-1283.

Elibol, O.H., D.D. Morisette, J.P. Denton and R. Bashir. 2003. Integrated nanoscale silicon sensors using top-down fabrication. **Appl. Phys. Lett., 83**: 4613–4615.

Elumalai, E.K and P. Vinothkumar. 2013. Role of silver nanoparticle against plant pathogens. **Nano Biomed. Eng., 5(2)**: 90-93.

Elumalai, E.K., T.N. Prasad, V. Kambala, P.C. Nagajyothi and E. David. 2010. Green synthesis of silver nanoparticle using *Euphorbia hirta* L. and their antifungal activities. **Arch. Appl. Sci. Res., 2 (6):** 76-81.

Fazaludeena, M.F., C. Manickamb, M.A.I. Ashankytyc, M.Q. Ahmedd and Q. Z. Beg. 2012. Synthesis and characterizations of gold nanoparticles by *Justicia gendarussa* Burm F. leaf extract. **J. Microbiol. Biotech. Res., 2**: 23-34.

Fesharaki, P.J., P. Nazari, M. Shakibaie, S. Rezaie and M. Banoee. 2010. Biosynthesis of selenium nanoparticles using *Klebsiella pneumoniae* and their recovery by a simple sterilization process. **Braz. J. Microbiol., 41**: 461-466.

Gade, A.K., P. Bonde, A.P. Ingle, P.D. Marcato and N. Duran. 2008. Exploitation of *Aspergillus niger* for synthesis of silver nanoparticles. **Journal of Biobased Materials and Bioenergy, 2**: 243-247.

Gajbhiye, M., J. Kesharwani, A. Ingle, A. Gade and M. Rai. 2009. Fungus mediated synthesis of silver nanoparticles and its activity against pathogenic fungi in combination of fluconazole. **Nanomedicine, 5(4)**: 282–286.

Ganesh Babu, M.M and P. Gunasekaran. 2009. Production and structural characterization of crystalline silver nanoparticles from *Bacillus cereus* isolate. **Colloids Surf: B Biointerfaces, 74**: 191-195.

Garg, S. 2012. Rapid biogenic synthesis of silver nanoparticles using black pepper (*Piper nigrum*) corn extract. **Int. J. Innov. Biol. Chem. Sci., 3**: 5-10.

Gavanji, S., M. Shams, N. Shafagh, A. Jalali, B. Larki, M. Doost Mohammadi, A.H. Taraghian, and S.V. Niknezhad. 2012. Destructive effect of silver nanoparticles on biocontrol agent fungi *Trichoderma viride* and *T. harzianum*. **Caspian Journal of Applied Sciences Research, 1(12)**: 83-90.

Gavhane, A.J., P. Padmanabhan, S.P. Kamble and S.N. Jangle. 2012. Synthesis of silver nanoparticles using extract of neem leaf and triphala and evaluation of their antimicrobial activities. **International Journal of Pharma and Biosciences, 3(3)**: 88 – 100.

Geethalakshmi, R and D.V.L. Sarada. 2010. Synthesis of plant mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their antimicrobial activities. **International Journal of Engineering Science and Technology, 2(5)**: 970-975.

Gengan, R.M., K. Anand, A. Phulukdaree and A. Chuturgoon. 2013. A549 lung cell line activity of biosynthesized silver nanoparticles using *Albizia adianthifolia* leaf. **Colloids and Surfaces B: Biointerfaces, 105**: 87-91.

Geoprincy, G., P. Saravanan, G.N. Nagendra and S. Renganathan. 2011. A novel approach of studying the combined antimicrobial effects of silver nanoparticles and antibiotics through agar over layer method and disc diffusion method. **Dig. J. Nanomat. Bios., 6(4):** 1557-1565.

Ghazvini, K., E. M. Hesabi and M.M. Akbarein. 2008. Antibacterial activity of a malodor neutralizer containing silver nanoparticles. **Journal of Cell and Molecular Research 1(1):** 47-50.

Ghormade, V., M.V. Deshpande and K.M. Paknikar. 2011. Perspectives for nano biotechnology enabled protection and nutrition of plants. **Biotechnol. Adv., 29**: 792–803.

Ghosh, S., S. Patil, M. Ahire, R. Kitture, S. Kale, K. Pardesi, S.S. Cameotra, J. Bellare, D.D. Dhavale, A. Jabgunde and A.B. Chopade. 2012. Synthesis of silver nanoparticles using *Dioscorea bulbifera* tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. **Int. J. Nanomed., 7**: 483- 496.

Gnanajobitha, G., G. Annadurai and C. Kannan. 2012. Green synthesis of silver nanoparticles using *Elettaria cardamomom* and assessment of its antimicrobial activity. **Int. J. Pharma Sci. Res., 3**: 323-330.

Gopalakrishnan, K., C. Ramesh, V. Ragunathan and M. Thamilselvan. 2012. Antibacterial activity of copperoxide nanoparticles on *Escherichia coli* synthesized from *Tridax procumbens* leaf extract and surface coating with poly- aniline. **Dig. J. Nanomater Bios., 7**: 833-839.

Gopinath, V., D. MubarakAli, S. Priyadarshini, M.N. Priyadharsshini, N. Thajuddin and P. Velusamy. 2012. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: A novel biological approach. **Colloids and Surfaces B: Biointerfaces, 96**: 69-74.

Govender, Y., T. Riddin, M. Gericke and C.G. Whiteley. 2009. Bioreduction of platinum salts into nanoparticles: A mechanistic perspective. **Biotechnol. Lett., 31**: 95–100.

Gu, H., P.L. Ho, E. Tong, L. Wang and B. Xu. 2003. Presenting vancomycin on nanoparticles to enhance antimicrobial activities. **Nano. Lett., 3**: 1261-1263.

Guidelli, E.J., A.P. Ramos, A.E.D. Zaniquelli and O. Baffa. 2011. Green synthesis of colloidal silver nanoparticles using natural rubber latex extracted from *Hevea brasiliensis*. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 82**: 140– 145.

Gurunathan, S., K. Kalishwaralal, R. Vaidyanathan, D. Venkataraman and S.R.K. Pandian. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. **Colloids Surf. B: Biointerfaces, 74**: 328-335.

Gutierrez, F.M., P.L. Olive, A. Banuelos, E. Orrantia, N. Nino, E.M. Sanchez, F. Ruiz, H. Bach and Y.A. Gay. 2010. Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. **Nanomedicine, 6**: 681–688.

Guzman, M., J. Dille and S. Godet. 2012. Synthesis and antibacterial activity of silver nano particles against gram positive and gram negative bacteria. **Nanomedicine: Nanotechnology, Biology and Medicine, 8**: 37-45.

Hainfeld, J.F and F.R. Furuya. 1992. A 1.4-nm gold cluster covalently attached to antibodies improves immune labeling. **J. Histochem. Cytochem., 40**: 177-184.

Hamouda, T and J.R. Baker. 2000. Antimicrobial mechanism of action of surfactant lipid preparations in enteric gram negative bacilli. **Journal of Applied Microbiology, 89**: 397-403.

Hassan, K., Z. Asharia, S. Iravanib and S. Abbasic. 2013. Optimization of biological synthesis of silver nanoparticles using *Fusarium oxysporum*. **Iranian Journal of Pharmaceutical Research, 12(3):** 289-298.

He, L., Y. Liu, A. Mustapha and M. Lin. 2011. Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. **Microbiol. Res., 166**: 207-215.

He, Y., Z. Du, H. Lv, Q. Jia, Z. Tang, X. Zheng, K. Zhang and F. Zhao. 2013. Green synthesis of silver nanoparticles by *Chrysanthemum morifolium* extract and their application in clinical ultrasound gel. **Int. J. Nanomedicine, 8:** 1809–1815.

Heald, C.M., B.D. Bruton and R.M. Davis. 1989. Influence of *Glomus intraradices* and soil phosphorus on *Meloidogyne incognita* in *Cucumis melo.* **Journal of Nematology, 21:** 69-73.

Hu, Y., G. Shen, H. Zhu and G. Jiang. 2010. A class-specific enzyme linked immunosorbent assay based on magnetic particles for multi residue organophosphorus pesticides. **Journal of Agricultural and Food Chemistry, 58:** 2801–2806.

Hudlikar, M., S. Joglekar, M. Dhaygude and K. Kodam. 2012. Latex mediated synthesis of ZnS nanoparticles: Green synthesis approach. **J. Nano. Res., 14**: 1- 6.

Hussain, Z and B.C. Bora. 2009. Interrelationship of *Meloidogyne incognita* and *Ralstonia solanacearum* complex in brinjal. **Indian Journal of Nematology, 39(1):** 41-45.

Husseiny, M.I., M.A. El-Aziz, Y. Badr and M.A. Mahmoud. 2007. Biosynthesis of gold nanoparticles using *Pseudomonas aeruginosa*. **Spectrochimica Acta A: Mol. Biomol. Spectrosc., 67**: 1003-1006.

Hwang, E.T., J.H. Lee, Y.J. Chae, Y.S. Kim, B.C. Kim, B.I. Sang and M.B. Gu. 2008. Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. **Small, 4:** 746-750.

Inbathamizh, L., T. Mekalai Ponnu and E. Jancy Mary. 2013. *In vitro* evaluation of antioxidant and anticancer potential of *Morinda pubescens* synthesized silver nanoparticles. **Journal of Pharmacy Research, 1**: 32-38.

Ingle, A., M. Rai, A. Gade and M. Bawaskar. 2009. *Fusarium solani*: A novel biological agent for the extracellular synthesis of silver nanoparticles. **J. Nanopart. Res., 11**: 2079-2085.

Ismail, O., M. Paret, M. Arslan, S. Kunwar, T. Chen, M. You and W. Tan. 2013. Nanotechnology in plant disease management: DNA-directed silver nanoparticles on graphene oxide as an antibacterial against *Xanthomonas perforans*. **ACS Nano., 7(10)**: 8972–8980.

Jacobs, C and R.H. Muller. 2002. Production and characterization of a budesonide nano suspension for pulmonary administration. **Pharmaceutical Research, 19**: 189–194.

Jagtap, U.B and V.A. Bapat. 2013. Green synthesis of silver nanoparticles using *Artocarpus heterophyllus* Lam. seed extract and its antibacterial activity. **Industrial Crops and Products, 46**: 132– 137.

Jain, D., H.K. Daima, S. Kachhwaha and S.L. Kothari. 2009. Synthesis of plant mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities. **Dig. J. Nanomater. Bios., 4**: 557- 563.

Jain, D., S. Kachhwaha, R. Jain, G. Srivastava and S.L. Kothari. 2010. Novel microbial route to synthesize silver nanoparticles using spore crystal mixture of *Bacillus thuringiensis*. **Indian J. Exp. Biol., 48**: 1152-1156.

Jebakumar, I.E.T and M.G. Sethuraman. 2012. Instant green synthesis of silver nanoparticles using *Terminalia chebula* fruit extract and evaluation of their catalytic activity on reduction of methylene blue. **Process Biochemistry, 47**: 1351-1357.

Jebakumar, I.E.T and M.G. Sethuraman. 2013. Biogenic robust synthesis of silver nanoparticles using *Punica granatum* peel and its application as a green catalyst for the reduction of anthropogenic pollutant 4-nitrophenol. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 104:** 262-264.

Jenkins, R. and R.L. Snyder. 1996. **Introduction to X-ray Powder Diffractiometry**. John Wiley and Sons. New York. p. 544.

Jha, A.K., K. Prasad and D. Prasad. 2009. A green low-cost biosynthesis of Sb20 nanoparticles. **Biochem. Eng. J., 43**: 303-306.

Jo, Y.K and B.H. Kim. 2009. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. **Plant Disease, 93 (10):** 1037 – 1043.

Joglekar, S., K. Kodam, M. Dhaygude and M. Hudlikar. 2011. Novel route for rapid biosynthesis of lead nanoparticles using aqueous extract of *Jatropha curcas* L. latex. **Mater. Lett., 5**: 3170–3172.

Joseph, T and M. Morrison. 2006. Nanotechnology in agriculture and food. **Nanoforum Report,** Institute of Nanotechnology. Pp: 1-13.

Juibari, M.M., S. Abbas alizadeh, G.S. Jouzani and M. Noruzi. 2011. Intensified biosynthesis of silver nanoparticles using a native extremophilic *Ureibacillus thermosphaericus* strain. **Mater. Lett., 65**: 1014-1017.

Jun, K.K., W.S. Sung, S.K. Moon, J.S. Choi, J.G. Kim and D.G. Lee. 2008. Antifungal effect of silver nanoparticles on dermatophytes. **J. Microbiol. Biotechnol., 18(8)**: 1482–1484.

Jung, J.H. S.W. Kim, J.S. Min, Y.J Kim, K. Lamsal, K.S Kim and Y.S Lee. 2010. The effect of nanosilver liquid against the white rot of the green onion caused by *Sclerotium cepivorum.* **Mycobiology, 38(1)**: 39-45.

Kalimuthu, K., R.S. Babu, D. Venkataraman, B. Mohd and S. Gurunathan. 2008. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. **Colloids Surf B: Biointerfaces, 65**: 150–153.

Kalishwaralal, K., S. Gurunathan, R. Vaidyanathan, D. Venkataraman, S.R.K. Pandian, J. Muniyandi, N. Hariharan and S.H. Eom. 2009. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. **Colloids Surf B: Biointerfaces, 74(1)**: 328–335.

Kamaraj, C., G. Rajakumar, A.A. Rahuman, K. Velayutham, A. Bagavan, A.A. Zahir and G. Elango. 2012. Feeding deterrent activity of synthesized silver nanoparticles using *Manilkara zapota* leaf extract against the house fly, *Musca domestica* (Diptera: Muscidae). **Parasitology Research, 111**: 2439–2448.

Kannan, N., K.S. Mukunthana and S. Balaji. 2011. Comparative study of morphology, reactivity and stability of synthesized silver nanoparticles using *Bacillus subtilis* and *Catharanthus roseus* (L.) G. Don. **Colloids and Surfaces B: Biointerfaces, 86:** 378-383.

Kaveri, K. 2014. Effect of nanomaterials in the management of *Sitophilus oryzae* L. in stored maize. **M.Sc. Thesis**, Tamil Nadu Agriculture University. Coimbatore. p.148.

Kavitha, K.S., S. Baker, D. Rakshith, H.U. Kavitha, H.C. Yashwantha Rao, B.P. Harini and S. Satish. 2013. Plants as green source towards synthesis of nanoparticles. **International Research Journal of Biological Sciences, 2(6)**: 66-76.

Kaviya, S., J. Santhanalakshmi and B. Viswanathan. 2011a. Green synthesis of silver nanoparticles using *Polyalthia longifolia* leaf extract along with D-Sorbitol: Study of antibacterial activity. **J. Nanotechnol., 5:** 123-137.

Kaviya, S., J. Santhanalakshmia, B. Viswanathan, J. Muthumary and K. Srinivasan. 2011b. Biosynthesis of silver nanoparticles using *Citrus sinensis* peel extract and its antibacterial activity. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 79**: 594-598.

Kawazoe, Y and J.A. Meech. 2005. Nanotechnology: Do good things really come in small packages. **Proceedings on 4th International Conference on Intelligent Processing and Manufacturing of Materials.** J. Meech, Y. Kawazoe, V. Kumar, J.F. Maguire (Eds.). DSE Tech Publications, Inc. Lancaster, PA, USA, Pp. 3-11.

Kelly, K.L., E. Coronado, L.L. Zhao and G.C. Schatz. 2003. The optical properties of metal nanoparticles: The influence of size, shape and dielectric environment. **Journal of Physical Chemistry, 107**: 668-677.

Kesharwani, J., K.Y. Yoon, J. Hwang and M. Rai. 2009. Phytofabrication of silver nanoparticles by leaf extract of *Datura metel*: Hypothetical mechanism involved in synthesis. **J. Bionanosci., 3**: 39-44.

Khalil, M.M.H., E.H. Ismail, K.Z.E. Baghdady and D. Mohamed. 2013. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. **Arab. J. Chem., 13:** 321-330.

Khan, M., M. Khan, S.F. Adil, M.N. Tahir, W. Tremel, H.Z. Alkhathlan and H. Siddiqui. 2013. Green synthesis of silver nanoparticles mediated by *Pulicaria glutinosa* extract. **International Journal of Nanomedicine, 8:** 1507–1516.

Khan, M.R and T. F. Rizvi. 2014. Nanotechnology: Scope and application in plant disease management. **Plant Pathology Journal, 13 (3):** 214-231.

Ki Jo, Y and B.H. Kim. 2009. Antifungal activity of silver ions and nanoparticles on phytopathogenic fungi. **Plant Disease, 93(10)**: 1037- 1043.

Kim, J.S., E. Kuk, K.N. Yu, J.H. Kim, S.J. Jin Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeong and M.H. Cho. 2007. Antimicrobial effects of silver nanoparticles. **Nanomedicine: Nanotechnology, Biology and Medicine, 3:** 95– 101.

Kim, K.J., W.S. Sung, S.K. Moon, J.S. Choi, J.G. Kim and D.G. Lee. 2008. Antifungal effect of silver nanoparticles on dermatophytes. **J. Microbiol. Biotechnol., 18**: 1482-1484.

Kim, S.W., J.H Jung, K. Lamsal, Y.S. Kim, J.S Min and Y.S Lee. 2012. Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. **Mycobiology, 40(1)**: 53-58.

Kirankumar, R., K.S. Jagadeesh, P.U. Krishnaraj and M.S. Patil. 2008. Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen. **Karnataka Journal of Agricultural Science, 21(2)**: 309-311.

Kiruba Daniel, S.C.G., R. Kumar, V. Sathish, M. Sivakumar, S. Sunitha and T. Anitha Sironmani. 2011. Green synthesis (*Ocimum tenuiflorum*) of silver nanoparticles and toxicity studies in zebra fish (*Danio rerio*) model. **Int. J. Nano Sci. Nanotechnol., 2 (2):** 103-117.

Klaus, T., R. Joerger, E. Olsson and C.G. Granqvist. 1999. Silver-based crystalline nanoparticles, microbially fabricated. **Proc. Natl. Acad. Sci. USA., 96**: 13611- 13614.

Kora, A.J., R.B. Sashidhar and J. Arunachalam. 2012. Aqueous extract of gum olibanum (*Boswellia serrata*): A reductant and stabilizer for the biosynthesis of antibacterial silver nanoparticles. **Process Biochemistry, 47:** 1516–1520.

Kowshik, M., S. Ashtaputre, S. Kharrazi, W. Vogel and J. Urban. 2003. Extracellular synthesis of silver nanoparticles by a silver tolerant yeast strain MKY3. **Nanotechnology, 14**: 95-100.

Krishnaraj, C., E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan and N. Mohan. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaves extracts and its antibacterial activity against waterborne pathogens. **Colloids Surf B: Biointerfaces, 76**: 50–56.

Krishnaraj, C., R. Ramachandran, K. Mohan and P.T. Kalaichelvan. 2012. Optimization for rapid synthesis of silver nanoparticles and its effect on phytopathogenic fungi. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 93**: 95– 99.

Kucharska, K., E. Pezowicz, D. Tumialis, D. Kucharski, B. Zajdel. 2009. The effect of silver nanoparticles on mortality and pathogenicity of entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) from Nematop biopreparation. **Animal Science, 48**: 21-23.

Kucharska, K., E. Pezowicz, D. Tumialis, D. Kucharski, B. Zajdel. 2014. Nanoparticles of silver and copper on entomopathogenic nematodes *Steinernema feltiae* (Filipjev, 1934) in reducing the number of the lesser mealworm beetle *Alphitobius diaperinus* (Panzer, 1797). **Animal Science, 53**: 29–35.

Kumar, C.G and S.K. Mamidyala. 2011. Extracellular synthesis of silver nanoparticles using culture supernatant of *Pseudomonas aeruginosa*. **Coll. Surf. B Biointerfaces, 84**: 462–466.

Kumar, P.K., P.M. Vemula, G. Ajayan and D. John. 2008. Silver nanoparticles embedded antimicrobial paints based on vegetable oil. **Nat. Mater., 7**: 236-241.

Kumar, R., S.M. Roopan, A. Prabhakarn, V.G. Khanna and S. Chakroborty. 2012. Agricultural waste *Annona squamosa* peel extract: Biosynthesis of silver nanoparticles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 90**: 173- 176.

Kwak, S.Y., S.H. Kim and S.S. Kim. 2001. Hybrid organic/inorganic reverse osmosis (RO) membrane for bactericidal anti-fouling. Preparation and characterization of TiO_2 nanoparticle self assembled aromatic polyamide thin-film-composite (TFC) membrane. **Environ. Sci. Technol., 35**: 2388-2394.

Lakshmi, Y.S.G. 2011. Green synthesis of silver nanoparticles from *Cleome Viscosa*: Synthesis and antimicrobial activity. **International Conference on Bioscience, Biochemistry and Bioinformatics**. Singapore. Pp: 334-337.

Lamsal, K., S.W. Kim, J.H. Jung, Y.S. Kim, K.S Kim and Y.S. Lee. 2011. Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. **Mycobiology, 39(3)**: 194-199.

Lengke, M., M.E. Fleet and G. Southam. 2006. Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold (I)–thiosulfate and gold (III)–chloride complexes. **Langmuir, 22**: 2780–2787.

Lim, D.Y., Y. Ji-Yeon Roh, Y. Hyun-Jeong Eom, Z. Jeong-Yun Choi, S. Jinwon Hyun and Jinhee Choi. 2012. Oxidative stress-related PMK-1 p38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode *Caenorhabditis elegans*. **Environmental Toxicology and Chemistry, 31(3)**: 585–592.

Linga Rao, M and N. Savithramma. 2012. Antimicrobial activity of silver nanoparticles synthesized by using stem extract of *Svensonia hyderobadensis* (Walp.) Mold- A rare medicinal plant. **Research in Biotechnology, 3(3)**: 41-47.

Liu, Y., L. He, A. Mustapha, H. Li and M. Lin. 2009. Antibacterial activities of zinc oxide nanoparticles against *Escherichia coli* O157:H7. **J. Appl. Microbiol., 107**: 1193-1201.

Logeswari, P., S. Silambarasan and J. Abraham. 2015. Synthesis of silver nanoparticles using plants extract and analysis of their antimicrobial property. **Journal of Saudi Chemical Society, 19(3)**: 311–317

Lok, C.N., C.M. Ho, R. Chen, Q.Y He, W.Y. Yu , H. Sun , P.K. Tam , J.F. Chiu and C.M. Che. 2007. Silver nanoparticles: partial oxidation and antibacterial activities. **J. Biol. Inorg. Chem., 12**: 527–534.

Lukman, A.I., B. Gong, C.E. Marjo, U. Roessner and A.T. Harris. 2011. Facile synthesis, stabilization and antibacterial performance of discrete AgNPs using *Medicago sativa* seed exudates. **Journal of Colloid and Interface Science, 353**: 433–444.

Lyons, K and G. Scrinis. 2009. Under the regulatory radar nanotechnologies and their impacts for rural Australia. In: **Tracking Rural Change: Community, Policy and Technology in Australia**, New Zealand and Europe, Merlan, F. (Eds.). Australian National University E Press, Canberra. Pp: 151-171.

Mahendra, R., Y. Alka and G. Aniket. 2009. Silver nanoparticles as a new generation of antimicrobials. **Biotechnology Advances, 27**: 76–83.

Maheswari, R.U., A.L. Prabha, V. Nandagopalan and V. Anburaja. 2012. Green synthesis of silver nanoparticles by using rhizome extract of *Dioscorea oppositifolia* L. and their antimicrobial activity against human pathogens**. IOSR J. Pharma. Biol. Sci., 1**: 38- 42.

Mahmood Chahardooli, Ehsan Khodadadi and Ehsaneh Khodadadi. 2014. Green synthesis of silver nanoparticles using oak (*Quercus infectoria*) leaf and fruit extracts and its antibacterial activity against plant pathogenic bacteria. **International Journal of Biosciences, 4(3)**: 97-103.

Mahtab, R., J.P. Rogers and C.J. Murphy. 1995. Protein sized quantum dot luminescence can distinguish between straight", "bent" and "kinked" oligonucleotides. **J. Am. Chem. Soc., 117**: 9099-9100.

Mallikarjuna, K., G.R. Dillip, G. Narashima, N.J. Sushma and B.D.P. Raju. 2012. Phytofabrication and characterization of silver nanoparticles from *Piper betel* broth. **Res. J. Nanosci. Nanotech., 2**: 17-23.

Mansoori, G.A. 2005. **Principles of Nanotechnology- molecular based study of condensed matter in small systems**, World Scientific Pub. Co., Hackensack, New Jersy, London. p.5.

Margarita Stoytcheva. 2011. **Pesticides Formulations, Effects, Fate**. In Tech. Rijeka, Croatia. Pp: 410-417.

Marimuthu, S., A.A. Rahuman, T. Santhoshkumar, C. Jayaseelan, A.V. Kirthi, A. Bagavan, C. Kamaraj, G. Elango, A.A. Zahir, G. Rajakumar and K. Velayutham. 2012. Lousicidal activity of synthesized silver nanoparticles using *Lawsonia inermis* leaf aqueous extract against *Pediculus humanus capitis* and *Bovicola ovis*. **Parasitology Research, 111**: 2023–2033.

Martinez, F., G. Peggy, L. Olive, A. Banuelos, E. Orrantia, N. Nino, E.M Sanchez, F. Ruiz, Y.A. Gay. 2010. Synthesis, characterization and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. **Nanomedicine: Nanotechnology, Biology and Medicine, 6**: 681–688.

Mary, E.J and L. Inbathamizh. 2012. Green synthesis and characterization of nano silver using leaf extract of *Morinda pubescens*. **Asian. J. Pharm. Clin. Res., 5**: 159-162.

Mason, C., S. Vivekanandhan, M. Misra and A.K. Mohanty. 2012. Switchgrass (*Panicum virgatum*) extract mediated green synthesis of silver nanoparticles. **World Journal of Nano Science and Engineering, 2**: 47-52.

McBeth, C.W., A.L. Taylor and A.L. Smith. 1941. Note on staining nematodes in root tissues. **Proceedings of the Helminthological Society of Washington, 16**: 3–6.

Mew, T.W, A.M. Alvarez, J.E. Leach and J. Swings. 1993. Focus on bacterial blight of rice. **Plant Disease, 77:** 5–12.

Meyer, J.N., C.A. Lord, X.Y. Yang, E. Turner, A.R. Badireddy, S.M. Marinakos, A. Chilkoti, M.R. Wiesner and M. Auffan. 2010. Intracellular uptake and associated toxicity of silver nanoparticles in *Caenorhabditis elegans*. **Aquatic Toxicology, 100**: 140–150.

Min, J.S., K.S. Kim, S.W. Kim, J.H. Jung, K. Lamsal, S.B. Kim, M. Jung and Y.S. Lee. 2009. Effects of colloidal silver nanoparticles on sclerotium forming phytopathogenic fungi. **Plant Pathol. J., 25(4)**: 376-380.

Mohanpuria, P., N.K. Rana and S.K. Yadav. 2007. Biosynthesis of nanoparticle, technological concepts and future application. **Journal of Nanoparticle Research, 7:** 75-79.

Mondal, S., N. Roy, R.A. Laskar, I. Sok, S. Basu, D. Mandal and N.A. Begum. 2011. Biogenic synthesis of Ag, Au and bimetallic Au/Ag alloy nanoparticles using aqueous extract of mahogany (*Swietenia mahogani*) leaves. **Colloids Surf B: Biointerfaces, 82**: 497-504.

Morones, J.R., J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Ramfrez and M.J. Yacaman. 2005. The bactericidal effect of silver nanoparticles. **Nanotechnology, 16**: 2346–2353.

Mourato, A., M. Gadanho, A.R. Lino and R. Tenreiro. 2011. Biosynthesis of crystalline silver and gold nanoparticles by extremophilic yeasts. **Bioinorg. Chem. Appl., 12:** 5460-5474.

Mubarak Ali, D., N. Thajuddin, K. Jeganathan and M. Gunasekaran. 2011. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. **Colloids and Surfaces B: Biointerfaces, 85**: 360-365.

Nabikhan, A., K. Kandasamy, A. Raj and N.M. Alikunh. 2010. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum*. **Colloids and Surfaces B: Biointerfaces, 79**: 488-493.

Nagati, V.B., J. Alwala, R. Koyyati, M.R. Donda, R. Banala and P.R.M. Padigya. 2012. Green synthesis of plant mediated silver nanoparticles using *Withania somnifera* leaf extract and evaluation of their antimicrobial activity. **Asian Pacific Journal of Tropical Biomedicine, 14:**1-5.

Nair, R., S.H. Varghese, B.G. Nair, T. Maekawa, Y. Yoshida and D.S. Kumar. 2010. Nanoparticulate material delivery to plants. **Plant Science, 179(3)**: 154–163.

Nanda, A and M. Saravanan. 2009. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE. **Nanomedicine, 5**: 452-456.

Narayanan, K.B and H.H. Park. 2014. Antifungal activity of silver nanoparticles synthesized using turnip leaf extract (*Brassica rapa* L.) against wood rotting pathogens. **Eur. J. Plant. Pathol., 65**: 1-8.

Nithya, R and R. Ragunathan. 2009. Synthesis of silver nanoparticles using *Pleurotus sajorcaju* and its antimicrobial study. **Digest Journal of Nanomaterials and Biostructures, 4**: 623-629.

Ondari, N.E and M.P. Nalini. 2014. Antimicrobial activity of biogenic silver nanoparticles synthesized using *Tridax procumbens* L. **Int. J. Curr. Res. Aca. Rev., 2(7):** 32-40.

Ouda, S.M. 2014. Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternata* and *Botrytis cinerea.* **Research Journal of Microbiology, 9 (1)**: 34-42.

Pal, S., Y. Kyung and J. Myong Song. 2007. Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle: A study of the gram negative bacterium *Escherichia coli*. **Appl. Environ. Microbiol., 73**: 1712-1720.

Palaniselvam, K., A.A.J. Velanganni, S.N. Govindan and Karthi. 2012. Leaf assisted bioreduction of silver ions using leaves of *Centella asiatica* L. and its bioactivity. **E- Journal of Life Sci., 1**: 46-49.

Panacek, A., L. Kvitek, R. Prucek, M. Kolar, R. Vecerova, N. Pizurova, V.K. Sharma, T. Nevecna and R. Zboril. 2006. Silver colloid nanoparticles: Synthesis, characterization and their antibacterial activity. **J. Phys. Chem., 110**: 16248–16253.

Panse, W.W and Sukhatme. 1978. **Statistical Methods for Agricultural Workers**. I.C.A.R., New Delhi, India. p.157.

Pant, G., N. Nayak and G. Prasuna. 2013. Enhancement of antidandruff activity of shampoo by biosynthesized silver nanoparticles from *Solanum trilobatum* plant leaf. **Applied Nanoscience, 3:** 431-439.

Papaiah, S., T.E. Seshadri Goud, B.S. Devi Prasad, K. Vemana and G. Narasimha. 2014. Silver nanoparticles a potential alternative to conventional antifungal agents to fungal pathogens affecting crop plants. **International Journal of Nano Dimension, 5(2)**: 139-144.

Parasad, K.S., D. Pathak, A. Patel, P. Dalwadi, R. Prasad, P. Patel and K. Selvaraj. 2011. Biogenic synthesis of silver nanoparticles using *Nicotiana tabaccum* leaf extract and study of their antimicrobial effect. **Afr. J. Biotechnol., 10**: 8122–8130.

Parashar, V., R. Parashar, B. Sharma and A.C. Pandey. 2009. Parthenium leaf extract mediated synthesis of silver nanoparticles: A novel approach towards weed utilization. **Dig. J. Nanomater. Biostruct., 4(1)**: 45 – 50.

Parida, U.K., B.K. Bindhani and P. Nayak. 2011. Green synthesis and characterization of gold nanoparticles using onion (*Allium cepa*) extract. **World J. Nano Sci. Eng., 7**: 93–98.

Parikh, D.V., T. Fink and K. Rajasekharan. 2005. Antimicrobial silver/sodium carboxymethyl cotton dressings for burn wounds. **Textile Research Journal, 75(2)**: 134–138.

Parikh, R.Y., S. Singh, B.L.V. Prasad, M.S. Patole, M. Sastry and Y.S. Shouche. 2008. Extracellular synthesis of crystalline silver nanoparticles and molecular evidence of silver resistance from *Morganella* sp.: Towards understanding biochemical synthesis mechanism. **Chem. Biochem., 9**: 1415–1422.

Park, H.J., S.H. Kim, H.J. Kim and S.H. Choi. 2006. A new composition of nanosized silica-silver for control of various plant diseases. **Plant Pathol. J., 22**: 295–302

Patil, D.C., V.S. Patil, P.H. Borase, K.B. Salunke and B.R. Salunkhe. 2012. Larvicidal activity of silver nanoparticles synthesized using *Plumeria rubra* plant latex against *Aedes aegypti* and *Anopheles stephensi*. **Parasitol. Res., 110**: 1815- 1822.

Patolsky, F., G. Zheng and C.M. Lieber. 2006. Nanowire sensors for medicine and life sciences. **Nanomedicine, 1**: 51–65.

Paulkumar, K., G. Gnanajobitha, M. Vanaja, S. Rajeshkumar, C. Malarkodi, K. Pandian and G. Annadurai. 2014. *Piper nigrum* leaf and stem assisted green synthesis of silver nanoparticles and evaluation of its antibacterial activity against agricultural plant pathogens. **The Scientific World Journal, 34**: 1-3.

Pavani, K.V., T. Swati, V. Snehika, K. Sravya and M. Sirisha. 2012. Phytofabrication of lead nanoparticles using grape skin extract. **Int. J. Eng. Sci. Tech., 4**: 3376-3380.

Petit, C., P. Lixon and M.P. Pileni. 1993. *In situ* synthesis of silver nanocluster in AOT reverse micelles. **Journal of Physical Chemistry, 97**: 12974–12983.

Petla, R.K., S. Vivekanandhan, M. Misra, A.K. Mohanty and N. Satyanarayana. 2012. Soybean (*Glycine Max*) leaf extract based green synthesis of palladium nanoparticles. **J. Biomater Nanobiotech., 3**: 14-19.

Philip, D. 2011. *Mangifera indica* leaf assisted biosynthesis of well dispersed silver nanoparticles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 78**: 327–331.

Ponarulselvam, S., Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu and S. Thangamani. 2012. Synthesis of silver nanoparticles using leaves of *Catharanthus roseus* Linn. G. Don and their antiplasmodial activities. **Asian Pacific Journal of Tropical Biomedicine, 2**: 574-580.

Prabakar, K., P. Sivalingam, S.I.M. Rabeek, M. Muthuselvam, N. Devarajan, A. Arjunan, R. Karthick, M.M. Suresh and J.P. Wembonyama. 2013. Evaluation of antibacterial efficacy of phytofabricated silver nanoparticles using *Mukia scabrella* (Musumusukkai) against drug resistance nosocomial gram negative bacterial pathogens. **Colloids and Surfaces B: Biointerfaces, 104**: 282– 288.

Prabhu, D., C. Arulvasu, G. Babu, R. Manikandan and P. Srinivasan. 2013. Biologically synthesized green silver nanoparticles from leaf extract of *Vitex negundo* L. induce growth-inhibitory effect on human colon cancer cell line HCT15. **Process Biochemistry, 48**: 317-324.

Prakash, P., P. Gnanaprakasam, R. Emmanuel, S. Arokiyaraj and M. Saravanan. 2013. Green synthesis of silver nanoparticles from leaf extract of *Mimusops elengi* L. for enhanced antibacterial activity against multi drug resistant clinical isolates. **Colloids and Surfaces B: Biointerfaces, 108**: 255–259.

Prathna, T.C., N. Chandrasekaran, A.M. Raichur and A. Mukherjee. 2011. Kinetic evolution studies of silver nanoparticles in a bio-based green synthesis process. **Colloids and Surfaces A: Physicochemical and Engineering Aspects, 377**: 212-216.

Pulit, J., M. Banach, R. Szczygłowska and M. Bryk. 2013. Nanosilver against fungi. Silver nanoparticles as an effective biocidal factor. **Acta Biochimica Polonica, 60(4)**: 795–798.

Raffi, M., F. Hussain, T.M. Bhatti, J.I. Akhter, A. Hameed and M.M. Hasan. 2008. Antibacterial characterization of silver nanoparticles against *Escherichia coli* ATCC-15224. **J. Mater. Sci. Technol., 24(2)**: 192-196.

Rai, A., A. Singh, A. Ahmad and M. Sastry. 2006. Role of halide ions and temperature on the morphology of biologically synthesized gold nanotriangles. **Langmuir, 22**: 736-741.

Rai, M., A. Yadav and A. Gade. 2008. Current trends in phytosynthesis of metal nanoparticles. **Crit. Rev. Biotechnol., 28(4)**: 277–284.

Rai, M., A. Yadav and A. Gade. 2009. Silver nanoparticles as a new generation of antimicrobials. **Biotechnology Advances, 27(1)**: 76-83.

Raja, K., A. Saravanakumar and R. Vijayakumar. 2012. Efficient synthesis of silver nanoparticles from *Prosopis juliflora* leaf extract and its antimicrobial activity using sewage. **Spectro Acta Part A: Molecular and Biomolecular Spectroscopy, 97**: 490-494.

Rajakumar, G and A.A. Rahuman. 2011. Larvicidal activity of synthesized silver nanoparticles using *Eclipta prostrata* leaf extract against filariasis and malaria vectors. **Acta Tropica., 118** : 196–203.

Rajamanickam, K., S.S. Sudha, M. Francis, T. Sowmya, J. Rengaramanujam, P. Sivalingam and K. Prabakar. 2013. Microalgae associated *Brevundimonas* sp. MSK 4 as the nanoparticles synthesizing unit to produce antimicrobial silver nanoparticles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 113**: 10-14.

Rajesh, S., D.P. Raja, J.M. Rathi and K. Sahayaraj. 2012. Biosynthesis of silver nanoparticles using *Ulva fasciata* (Delile) ethyl acetate extract and its activity against *Xanthomonas campestris* pv. *malvacearum*. **J. Biopest., 5**: 119-128.

Ramamurthy, C.H., M.I. Padma, D.M. Samadanam, R. Mareeswaran, A. Uyavaran, M. Suresh Kumar, K. Premkumar and C. Thirunavukkarasu. 2013. The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. **Colloids and Surfaces B: Biointerfaces, 102**: 802-815.

Raman, N., S. Sudharsan, V. Veerakumar, N. Pravin and K. Vithiya. 2012. *Pithecellobium dulce* mediated extra-cellular green synthesis of larvicidal silver nanoparticles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 96**: 1031-1037.

Ramteke, C., T. Chakrabarti, B.K. Sarangi and R. Pandey. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum* for enhanced antibacterial activity. **J. Chem., 34**: 1-7.

Rang, A., L. Han, E.R. Kim, J. Kim, Y.S. Kim and Y. Park. 2012. Enhanced antibacterial activities of *Leonuri herba* extracts containing silver nanoparticles. **Phytother. Res., 26**: 1249-1255.

Ravikumar, P and S. Sathish Kumar. 2014. Antifungal activity of extracellularly synthesized silver nanoparticles from *Morinda citrifolia* L. **International Journal of Technical Research and Applications, 2(4):** 108-111.

Riker, A.J and R.S. Riker. 1936. **Introduction to research on plant disease**. John S Swift and Co. Inc., St. Louis, Chicago. Pp: 450-456.

Rodriques, A.G., L.Y. Ping, P.D. Marcato, O.L. Alves, M.C.P. Silva, R.C. Ruiz, I.S. Melo, L. Tasic and A.O. DeSouza. 2012. Biogenic antimicrobial silver nanoparticles produced by fungi. **Applied Microbiology Biotechnology, 97(2):** 775-782.

Roopan, S.M., G. Rohita, A. Madhumitha, A. Rahuman, C. Kamaraj, A. Bharathi and T.V. Surendra. 2013. Low cost and ecofriendly phytosynthesis of silver nanoparticles using *Cocos nucifera* coir extract and its larvicidal activity. **Industrial Crops and Products, 43**: 631-635.

Rouhani, M., M.A. Samih and S. Kalantari. 2012. Insecticide effect of silver and zinc nanoparticles against *Aphis nerii* (Hemiptera: Aphididae). **Chilean Journal of Agricultural Research, 72(4)**: 590 – 594.

Sable, N., S. Gaikwad, S. Bonde, A. Gade and M.M. Rai. 2012. Phytofabrication of silver nanoparticles by using aquatic plant *Hydrilla verticilata.* **Nus. Biosci., 4**: 45-49.

Safer, D., L. Bolinger and J.S. Leigh. 1986. Undecagold clusters for site specific labeling of biological macromolecules: simplified preparation and model applications. **J. Inorg. Biochem., 26**: 77-91.

Sahayaraja, K., S. Rajesh and J.M. Rathib. 2012. Silver nanoparticles biosynthesis using marine alga *Padina Pavonica* (Linn.) and its microbicidal activity. **Digest Journal of Nanomaterials and Biostructures, 7(4)**: 1557-1567

Sahu, N., D. Soni, B. Chandrashekhar, B.K. Sarangi, D. Satpute and R.A. Pandey. 2012. Synthesis and characterization of silver nanoparticles using *Cynodon dactylon* leaves and assessment of their antibacterial activity. **Bioprocess and Biosystems engineering, 36 (7):** 999-1004.

Salata, O.V. 2004. Application of nanoparticles in biology and medicine. **J. Nanobiotechnology, 2**: 13-20.

Sandhya, M., B.R. Singh, A. Singh, C. Keswani, A.H. Naqvi and H.B. Singh. 2014. Biofabricated silver nanoparticles act as a strong fungicide against *Bipolaris sorokiniana* causing spot blotch disease in wheat. **Plos One, 9(5)**: 1-11.

Sanghi, R and P. Verma. 2009. Biomimetic synthesis and characterization of protein capped silver nanoparticles. **Bioresource Technology, 100**: 501-504.

Santhosh kumar, T., A.A. Rahuman, A. Bagavan, S. Marimuthu, C. Jayaseelan, A. Vishnu Kirthi, C. Kamaraj, G. Rajakumar, A.A. Zahir, G. Elango, K. Velayutham, M. Iyappan, C. Siva, L. Karthik and V.B.K. Rao. 2012. Evaluation of stem aqueous extract and synthesized silver nanoparticles using *Cissus quadrangularis* against *Hippobosca maculata* and *Rhipicephalus microplus*. **Experimental Parasitology, 132**: 156-165.

Sasser, J.N. 1979. Economic importance of *Meloidogyne* in tropical countries. In: **Root knot nematodes (*Meloidogyne spp*.) systematics, biology and control.** Academic Press Inc. London, New York, USA. Pp: 120-125.

Sastry, M., A. Ahmad, M. Islam Khan and R. Kumar. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycetes. **Curr. Sci., 85**: 162–170.

Sathishkumar, M., K. Sneha, S.W. Won, C.W. Cho, S. Kim and Y.S. Yun. 2012. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nanocrystalline silver particles and its bactericidal activity. **Colloids Surfaces B: Biointerfaces, 73**: 332–338.

Satyavani, K., T. Ramanathan and S. Gurudeeban. 2011. Plant mediated synthesis of biomedical silver nanoparticles by using leaf extract of *Citrullus colocynthis*. **J. Nanosci. Nanotechno., 1**: 95-101.

Saxena, A., R.M. Tripathi, F. Zafar and P. Singh. 2012. Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antimicrobial activity. **Mater Letters, 67**: 91–94.

Schindler, A.F. 1961. A simple substitute for a Baermann funnel. **Plant Disease Reporter, 45:** 747:748.

Scrinis, G and K. Lyons. 2007. The emerging nano corporate paradigm nanotechnology and the transformation of nature, food and agrifood systems. **International Journal of Sociology Food Agriculture, 15(2)**: 22–44.

Seshadri, S., K. Saranya and M. Kowshik. 2011. Green synthesis of lead sulfide nanoparticles by the lead resistant marine yeast, *Rhodosporidium diobovatum*. **Biotechnol. Prog., 27**: 1464-1469.

Shah, S.N., N.F. Steinmetz, A.A. Aljabali, G.P. Lomonossoff and D.J Evans. 2009. Environmentally benign synthesis of virus template, monodisperse, iron plantinum nanoparticles. **Dalton Trans., 40**: 8479-8480.

Shahverdi, A.R., Ali Fakhimi, R. Hamid, R. Shahverdi and Sara Minaian. 2007. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against *Staphylococcus aureus* and *Escherichia coli*. **Nanomedicine: Nanotechnology, Biology and Medicine, 3**: 168–171.

Shaiu, W.L., D.D. Larson, J. Vesenka and E. Henderson. 1993. Atomic force microscopy of oriented linear DNA molecules labeled with 5 nm gold spheres. **Nucleic Acids Res., 21**: 99-103.

Shameli, K., M.B. Ahmad, E.A.J. Al Mulla, N.A. Ibrahim, P. Shabanzadeh, A. Rustaiyan, Y. Abdollahi, S. Bagheri, S. Abdolmohammadi, M.S. Usman and M. Zidan. 2012. Green biosynthesis of silver nanoparticles using *Callicarpa maingayi* stem bark extraction. **Molecules, 17**: 8506-8517.

Shankar, S.S., A. Rai, B. Ankamwar, A. Singh, A. Ahmad and M. Sastry. 2004. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. **Journal of Colloid and Interface Science, 275 (2):** 496-502

Sharma, V.K., R.A. Yngard and Y. Lin. 2009. Silver nanoparticles: green synthesis and their antimicrobial activities. **Advances in Colloid and Interface Science, 145**: 83–96.

Shavandi, Z., T. Ghazanfari, K. Nazari Moghaddam and E. Abdi Alaee. 2010. Investigation of inhibitory effects of colloidal silver nanoparticles on three bacterial species and macrophagous cells in 24 hours cultures. **Journal of Daneshvar Pezeshki, 17(88)**: 9-16.

Sheikpranbabu, S., K. Kalishwaralal, D. Venkataraman, S.H. Eom, J. Park and S. Gurunathan. 2009. Silver nanoparticles inhibit VEGF-and IL-1b-induced vascular permeability *viz.*, SRC dependent pathway in porcine retinal endothelial cells. **J. Nanobiotechnol., 7**: 8-10

Shenton, W., S.A. Davies and S. Mann. 1999. Directed self-assembly of nanoparticles into macroscopic materials using antibody antigen recognition. **Adv. Mater., 11**: 449-452.

Shenya, D.S., J. Joseph Mathew and D. Philip. 2011. Phytosynthesis of Au, Ag and Au–Ag bimetallic nanoparticles using aqueous extract and dried leaf of *Anacardium occidentale.* **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 79**: 254-262.

Shrivastava, S., T. Bera, A. Roy, Gajendra Singh, P. Ramachandrarao and D. Dash. 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. **Nanotechnology, 18**: 1-9.

Siddiqui, Z.A and S.I. Husain. 1992. Interaction of *Meloidogyne incognita* race 3 and *Macrophomina phaseolina* in root rot disease complex of chickpea. **Nematologia Mediterranea, 20(2):** 199-202.

Sileikaite, A., P. Judita, P. Igoris and T. Sigitas. 2009. Investigation of silver nanoparticles formation kinetics during reduction of silver nitrate with sodium citrate. **Materials Science, 15**: 21-27.

Singh, C., V. Sharma, K.R.P. Naik, V. Khandelwal and H. Singh. 2011. A green biogenic approach for synthesis of gold and silver nanoparticles using *Zingiber officinale*. **Dig. J. Nanomater. Bios., 6**: 535-542.

Singhal, G., R. Bhavesh, K. Kasariya, A.R. Sharma and R.P. Singh. 2011. Biosynthesis of silver nanoparticles using *Ocimum sanctum* (Tulsi) leaf extract and screening its antimicrobial activity. **J. Nanopart. Res., 13**: 2981–2988.

Sintubin, L., W. De Windt, J. Dick, J. Mast and D. Van der Ha. 2009. Lactic acid bacteria as reducing and capping agent for the fast and efficient production of silver nanoparticles. **Appl. Microbiol. Biotechnol., 84**: 741-749.

Sivakumar, P., C. Nethradevi and S. Renganathan. 2012. Synthesis of silver nanoparticles using *Lantana camara* fruit extract and its effect on pathogens. **Asian J. Pharm. Clin. Res., 5**: 97- 101.

Sondi, I and B. Salopek Sondi. 2004. Silver nanoparticles as antimicrobial agent: A case study on *Escherichia coli* as a model for gram-negative bacteria. **Journal of Colloids Interface Science, 75**: 177–182.

Soundarrajan, C., A. Sankari, P. Dhandapani, S. Maruthamuthu, S. Ravichandran, G. Sozhan and N. Palaniswamy. 2012. Rapid biological synthesis of platinum nanoparticles using *Ocimum sanctum* for water electrolysis applications. **Bioprocess Biosyst. Eng., 35**: 827-833.

Srivastava, M., S.K. Gupta, A.P. Saxena, L.A.J. Shittu and S.K. Gupta. 2011. A Review of occurrence of fungal pathogens on significant Brassicaceous vegetable crops and their control measures. **Asian J. Agric Sci., 3**: 70- 79.

Srivathsan, J., V. Sivakami, B. Ramachandran, K.S. Harikrishna, S. Vetriselvi and D.J. Mukesh Kumar. 2012. Synthesis of silver nanoparticles and its effect on soil bacteria. **J. Microbiol. Biotech. Res., 2(6)**: 871-874.

Storz, G. and J.A. Imlay. 1999. Oxidative stress. **Curr. Opin. Microbiol., 2:** 188-194.

Subba Rao, Y., V.S. Kotakadi, T.N.V.K.V. Prasad, A.V. Reddy and D.V.R. Sai Gopal. 2013. Green synthesis and spectral characterization of silver nanoparticles from Lakshmi tulsi (*Ocimum sanctum*) leaf extract. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 103**: 156–159.

Sukirtha, R., K.M. Priyanka, J.J. Antony, S. Kamalakkannan, R. Thangam, P. Gunasekaran, M. Krishnan and S. Achiraman. 2012. Cytotoxic effect of Green synthesized silver nanoparticles using *Melia azedarach* against *in vitro* HeLa cell lines and lymphoma mice model. **Process Biochemistry, 47**: 273–279.

Sulochana, S., P. Krishnamoorthy and K. Sivaranjani. 2012. Synthesis of silver nanoparticles using leaf extract of *Andrographis paniculata*. **J. Pharmacol. Toxicol., 7**: 251-258.

Suman, T.Y., S.R. Radhika Rajasree, A. Kanchana and S.B. Elizabeth. 2013. Biosynthesis, characterization and cytotoxic effect of plant mediated silver nanoparticles using *Morinda citrifolia* root extract. **Colloids and Surfaces B: Biointerfaces, 106**: 74-78.

Sumitra, C. 2013. Silver nanoparticles (medicinal plants mediated): A new generation of antimicrobials to combat microbial pathogens- A review. **Formatex, 13:** 1314-1323.

Sundaravadivelan, C and M. Nalini. 2012. Biolarvicidal effect of phytosynthesized silver nanoparticles using *Pedilanthus tithymaloides* (L.) Poit stem extract against the dengue vector *Aedes aegypti* L. (Diptera; Culicidae). **Asian. Pac. J. Trop. Biomed., 2:** 1-8.

Sweeney, R.Y., C. Mao, X. Gao, J.L. Burt and A.M. Belcher. 2004. Bacterial biosynthesis of cadmium sulfide nanocrystals. **Chem. Biol., 11**: 1553-1559.

Taylor, A.L and J.N. Sasser. 1978. Biology, **Identification and control of root knot nematodes (*Meloidogyne* spp.)** Croop. Publ. Deps. Plant Pathology, North Carolina State University and U.S. Agency for International Developement. Raliegh, North Carolina. p. 111.

Thakkar, K.N., S.S. Mhatre and R.Y. Parikh. 2010. Biological synthesis of metallic nanoparticles. **Nanomedicine, 6(2)**: 257–262

Thirumurugan, A., G.J. Jiflin, G. Rajagomathi, N.A. Tomy, S. Ramachandran and R. Jaiganesh. 2010. Biotechnological synthesis of gold nanoparticles of *Azadirachta indica* leaf extract. **Int. J. Biol. Tech., 1**: 75-77.

Thul, S.T., B.K. Sarangi and R.A. Pandey. 2013. Nanotechnology in agroecosystem: Implications on plant productivity and its soil environment. **Expert Opinion on Environmental Biology, 2**: 1-3.

Tripathy, A., M.A. Raichur, N. Chandrasekaran, T.C. Prathna and A. Mukherjee. 2010. Process variables in biomimetic synthesis of silver nanoparticles by aqueous extract of *Azadirachta indica* (Neem) leaves. **Journal of Nano Research, 12(1)**: 237-246.

Usha Rani, P and P. Rajasekharreddy. 2011. Green synthesis of silver-protein (core–shell) nanoparticles using *Piper betle* L. leaf extract and its ecotoxicological studies on *Daphnia magna*. **Colloids and Surfaces A: Physicochemical and Engineering Aspects, 89**: 188-194.

Valodkar, M., P.S. Nagar, R.N. Jadeja, M.C. Thounaojam, R.V. Devkar and S. Thakore. 2011. Euphorbiaceae latex induced green synthesis of non-cytotoxic metallic nanoparticle solutions: A rational approach to antimicrobial applications. **Colloids and Surfaces A: Physicochemical and Engineering Aspects, 84**: 337-344.

Vanaja, M and G. Annadurai. 2012. *Coleus aromaticus* leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity. **Appl. Nanosci., 3:** 217–223

Vankar, P.S and D. Bajpai. 2010. Preparation of gold nanoparticles from *Mirabilis jalapa* flowers. **Indian J. Biochem. Biophys., 47**: 157- 160.

Veerasamy, R., T.Z. Xin, S. Gunasagaran, T.F. Xiang, E.F. Yang, N. Jeyakumar and S.A. Dhanaraj. 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. **Journal of Saudi Chemical Society, 15**: 113–120.

Vidhu, V.K., S.A. Aromal and D. Philip. 2011. Green synthesis of silver nanoparticles using *Macrotyloma uniflorum*. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 83**: 392-397.

Vijayakumar, M., K. Priya, F.T. Nancy, A. Noorlidah and A.B.A. Ahmed. 2013. Biosynthesis, characterization and anti-bacterial effect of plant mediated silver nanoparticles using *Artemisia nilagirica.* **Industrial Crops and Products, 41**: 235-240.

Wang, H., R. Wick, L. Xing and Baoshan. 2009. Toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode *Caenorhabditis elegans*. **Environ. Pollut., 157(4):** 1171-1177.

Wani, A.H and M.A. Shah. 2012. A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. **Journal of Applied Pharmaceutical Science, 2**: 40-44.

White, R.J., V.L. Budarin, J.W.B. Moir and J.H. Clark. 2011. A sweet killer: Mesoporous polysaccharide confined silver nanoparticles for antibacterial applications. **Int. J. Mol. Sci., 12**: 5782 - 5796.

Winstead, N.N and A. Kelman. 1952. Inoculation technique for evaluation of resistance to *Pseudomonas solanacearum*. **Phytopathology, 42**: 628-634.

Woo, K.S., K.S. Kim, K. Lamsal, Y.J. Kim, S.B. Kim, J.M. young, S.J. Sim, H.S. Kim, S.J. Chang, J.K. Kim and Y.S. Lee. 2009. An *in vitro* study of the antifungal effect of silver nanoparticles on oak wilt pathogen *Raffaelea* sp. **J. Microbiol. Biotechnol., 19(8)**: 760–764.

Xin, Y., L. Qingbiao, W. Huixuan, H. Jiale, L. Liqin, W. Wenta, S. Daohua, S. Yuanbo, O. James, H. Luwei, W. Yuanpeng, H. Ning and J. Lishan. 2010. Green synthesis of palladium nanoparticles using broth of *Cinnamomum camphora* leaf. **J. Nanopart. Res., 12**: 1589-1598.

Yamanaka, M., K. Hara and J. Kudo. 2005. Bactericidal actions of a silver ion solution on *Escherichia coli* studied by energy filtering Transmission Electron Microscopy and proteomic analysis. **Appl. Environ. Microbiol., 71**: 7589-7593.

Yan, I., K. Huang, Y. Wang and S. Liu. 2005. Study on antipollution nanopreparation of dimethomorph and its performance. **Chin. Sci. Bull., 50**: 108-112.

Yang, X., A.P. Gondikas, J.N. Meyer, S. M. Marinakos, M. Auffan, J. Liu and H.H. Kim. 2012. Mechanism of silver nanoparticle toxicity is dependent on dissolved silver and surface coating in *Caenorhabditis elegans*. **Environmental Science and Technology, 46**: 1119-1127.

Yehia, R.S and O.F. Ahmed. 2013. *In vitro* study of the antifungal efficacy of zinc oxide nanoparticles against *Fusarium oxysporum* and *Penicilium expansum*. **African Journal of Microbiology Research, 7(19)**: 1917-1923.

Yeon Roh, J., S.J. Sim, J. Yi, K. Park, K.H Chung, D.Y. Ryu and J.H Choi. 2009. Ecotoxicity of silver nanoparticles on the soil nematode *Caenorhabditis elegans* using functional ecotoxicogenomics. **Environ. Sci. Technol., 43**: 3933–3940.

Zaved, M.F., W.H. Eisa and A.A. Shabaka. 2012. *Malva parviflora* extract assisted green synthesis of silver nanoparticles. **Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 98**: 423-428.

Zhang, H., Q. Li, Y. Lu, D. Sun and X. Lin. 2005. Biosorption and bioreduction of diamine silver complex by Corynebacterium. **J. Chem. Technol. Biotechnol., 80**: 285-290.

Table 5. Comparison of characters of green synthesized AgNPs using different plants

S.No	Characters	Instruments used	Plants		
			Tridax procumbens	***Euphorbia hirta***	***Azardirachta indica***
1.	Particle size	Particle Size Analyzer	78.9 nm	87.6 nm	40.4 nm
2.	Particle stability	Zeta potential	-41.7 mV	-27.9mV	-37.2mV
3.	Confirmation of AgNPs synthesis	Uv-Vis Spectroscopy	Confirmed with absorbance peak at 435 nm	Confirmed with absorbance at 444 nm	Confirmed with absorbance at 400 nm
4.	Shape	SEM	Spherical with agglomeration		
		TEM	Spherical with size of 15.5 - 31.8 nm	Spherical with size of 35- 49 nm	Spherical with size of 26.7 - 43.2 nm
5.	Silver elemental composition (%)	SEM (EDAX)	97.91	86.83	78.12
6.	Nature of particles	XRD	Crystalline		

Table 6. Effect of green synthesized AgNPs *in vitro* on egg hatchability of *Meloidogyne incognita*

Concentrations of AgNPs	Inhibition in egg hatching (%) at different intervals (Days)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0.0005 %	85.72 (67.80)	86.94 (68.81)	89.44 (71.04)	90.43 (71.98)	90.72 (72.27)	90.97 (72.51)	91.49 (73.04)
0.0001%	79.97 (63.42)	82.08 (64.95)	85.31 (67.46)	86.42 (68.38)	86.67 (68.59)	87.01 (68.87)	87.35 (69.17)
0.00005 %	69.49 (56.47)	73.01 (58.70)	76.89 (61.27)	79.59 (63.14)	80.50 (63.80)	81.69 (64.67)	82.64 (65.38)
0.00001%	49.77 (44.87)	50.72 (45.41)	59.62 (50.55)	62.47 (52.22)	62.70 (52.86)	65.97 (54.31)	67.17 (55.04)
Untreated control	70.51	83.69	108.62	123.41	130.84	142.61	153.55
SEd	**0.47**	**0.72**	**0.61**	**0.51**	**0.65**	**0.73**	**0.82**
CD(P=0.05)	**1.00**	**1.54**	**1.32**	**1.09**	**1.38**	**1.56**	**1.74**

* Figures in parentheses are arcsine transformed values.

Table 7. Effect of green synthesized AgNPs *in vitro* on juveniles of *M. incognita*

Concentrations of AgNPs	Mortality of juveniles (%) at different intervals (Days)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0.0005 %	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
0.0001%	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
0.00005 %	90.42 (71.97)	93.15 (74.83)	95.43 (77.66)	96.73 (79.58)	98.54 (83.06)	100.00 (90.00)	100.00 (90.00)
0.00001%	20.35 (26.81)	25.74 (30.49)	28.17 (32.06)	30.52 (33.54	32.38 (34.68)	35.44 (36.54)	36.82 (37.36)
Untreated control	1.00 (5.74)	2.00 (8.13)	5.35 (13.37)	7.42 (15.81)	11.2 (19.55)	15.59 (23.26)	17.27 (24.51)
SEd	**1.12**	**0.74**	**0.78**	**0.93**	**0.49**	**1.14**	**0.78**
CD(P=0.05)	**2.4**	**1.58**	**1.66**	**1.99**	**1.03**	**2.45**	**1.67**

* Figures in parentheses are arcsine transformed values.

Table 8. Effect of green synthesized AgNPs on attraction of *M. incognita* juveniles towards roots of tomato *in vitro*

Concentrations of AgNPs	J_2 attracted at different intervals (h)				
	24h	**48h**	**72h**	**96h**	**120h**
0.0005 %	3.21 (94.67)	9.31 (87.27)	14.14 (82.83)	20.56 (77.10)	25.10 (74.15)
0.0001 %	5.34 (91.14)	12.56 (82.82)	26.34 (68.01)	43.11 (51.98)	50.01 (48.50)
0.00005 %	11.57 (80.79)	28.31 (61.28)	42.55 (48.33)	49.82 (44.50)	61.21 (36.97)
0.00001 %	24.61 (59.15)	39.78 (45.60)	44.67 (45.76)	52.12 (41.94)	66.74 (31.27)
Untreated control	60.24	73.12	82.35	89.77	97.11
SEd	**0.16**	**0.32**	**0.71**	**0.39**	**0.73**
CD (P=0.05)	**0.34**	**0.69**	**1.53**	**0.83**	**1.56**

* Figures in parentheses are per cent decrease over control.

Table 9. Effect of green synthesized AgNPs on root penetration of *M. incognita* juveniles in tomato

Concentrations of AgNPs	J_2 penetrated after inoculation (Days)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
0.0005%	5.15 (90.16)	7.44 (87.84)	9.51 (86.45)	12.10 (85.20)	15.54 (82.22)	17.55 (80.93)	19.20 (79.76)
0.0001 %	6.18 (88.19)	11.51 (81.18)	16.65 (76.27)	21.87 (73.25)	26.51 (69.88)	28.88 (68.17)	31.67 (66.62)
0.00005%	9.88 (81.12)	18.43 (71.01)	24.74 (64.74)	30.69 (62.46)	32.56 (62.47)	35.43 (60.67)	40.87 (56.92)
0.00001 %	18.44 (64.77)	25.66 (58.05)	32.12 (54.22)	36.21 (55.72)	44.33 (49.86)	48.10 (46.71)	55.01 (42.02)
Untreated control	52.34	61.17	70.16	81.77	87.42	89.98	94.87
SEd	**0.26**	**0.38**	**0.48**	**0.47**	**0.54**	**0.49**	**0.88**
CD(P=0.05)	**0.57**	**0.83**	**1.03**	**1.01**	**1.14**	**1.06**	**1.89**

* Figures in parentheses are per cent decrease over control.

Table 10. Effect of green synthesized AgNPs *in vitro* on mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*

Concentration of AgNPs (%)	Radial growth of the pathogen (mm)	Decrease over control (%)
0.001	17.00	81.11
0.005	15.00	83.33
0.010	13.00	85.56
0.025	10.85	87.94
0.050	5.25	94.17
0.075	4.00	95.56
0.100	3.00	96.67
Untreated control	90.00	-
SEd	**0.41**	
CD(P=0.05)	**0.89**	

Table 11. Effect of green synthesized AgNPs on mycelial growth of *Macrophomina phaseolina*

Concentrations of AgNPs (%)	Radial growth of the pathogen (mm)	% decrease over control
0.001	15.63	82.63
0.005	10.27	88.58
0.010	7.83	91.30
0.025	5.00	94.44
0.050	3.00	96.66
0.075	2.27	97.47
0.100	1.00	98.88
Untreated control	90.00	-
SEd	**0.84**	
CD(P=0.05)	**1.79**	

Table 12. Effect of green synthesized AgNPs on bacterial pathogens *in vitro*

Concentrations of AgNPs (%)	Zone of inhibition (mm)	
	Ralstonia solanacerarum	***Xanthomonas oryzae***
0.001	0.50 (-)	0.50 (-)
0.005	0.50 (-)	0.50 (-)
0.010	10.07 (95.03)	9.57 (94.76)
0.025	12.78 (96.08)	13.00 (96.15)
0.050	15.57 (96.79)	15.08 (96.69)
0.075	16.83 (97.02)	15.92 (96.85)
0.100	20.33 (97.54)	18.58 (97.30)
Untreated control	0.50	0.50
SEd	**0.05**	**0.02**
CD(P=0.05)	**0.11**	**0.04**

*Figures in parentheses are per cent increase over untreated control.

Table 13. Influence of green synthesized AgNPs on *M. incognita* in tomato under glasshouse conditions

Treatments	Nematode population		Females/ g root	Females with egg mass/g root	Eggs/ egg mass	Gall index
	Soil (250cc)	Root (5g)				
Green synthesized AgNPs at 0.0005%	35.60 (88.16)	7.55 (92.79)	5.98 (71.59)	2.68 (79.80)	101.21 (65.14)	0.2
Pseudomonas fluorescens @ 2.5kg/ha	61.67 (79.49)	21.18 (79.77)	13.74 (34.75)	7.89 (40.52)	110.45 (61.96)	1.0
Carbofuran @1kg a.i./ha	72.87 (75.77)	35.50 (66.10)	15.63 (25.77)	11.26 (15.15)	120.35 (58.55)	3.0
Untreated control	300.69	104.71	21.05	13.27	290.33	5.0
SEd	**1.55**	**0.36**	**0.27**	**1.23**	**1.40**	
CD (P=0.05)	**3.39**	**0.78**	**0.58**	**0.27**	**3.05**	

* Figures in parentheses are per cent decrease over control

Table 14. Influence of green synthesized AgNPs on plant growth characters of tomato challenged with *M. incognita* under glasshouse conditions

Treatments	Shoot		Root		Fruit yield /plant (g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.0005%	68.43 (26.99)	152.35 (13.92)	37.90 (26.57)	30.88 (23.45)	103.45 (20.54)
P. fluorescens @ 2.5kg/ha	67.16 (25.61)	149.09 (12.03)	35.06 (20.62)	28.79 (17.89)	97.45 (15.65)
Carbofuran @ 1Kg a.i./ha	57.50 (13.11)	140.25 (6.49)	31.25 (10.94)	26.95 (12.27)	92.06 (10.71)
Untreated control	49.96	131.15	27.83	23.64	82.20
SEd	**1.84**	**1.40**	**1.47**	**0.94**	**1.89**
CD (P=0.05)	**4.01**	**3.07**	**3.22**	**2.05**	**4.14**

* Figures in parentheses are per cent increase over control

Table 15. Influence of green synthesized AgNPs in tomato inoculated with *F. oxysporum* f.sp. *lycopercsici* under glasshouse conditions

Treatments	Shoot		Root		PDI	Fruit yield /plant(g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)		
Green synthesized AgNPs at 0.1%	69.91 (21.74)	120.34 (21.51)	36.71 (29.99)	25.39 (26.81)	25.00 (60.52)	96.45 (14.77)
P. fluorescens @ 2.5kg/ha	67.53 (18.98)	118.94 (20.59)	34.78 (26.11)	23.23 (20.01)	44.33 (30.00)	93.45 (12.04)
Carbendazim at 0.1%	59.96 (8.76)	100.88 (6.37)	29.15 (11.84)	20.82 (10.75)	46.83 (26.05)	90.06 (8.73)
Untreated control	54.71	95.45	25.70	18.58	63.33	82.20
SEd	**0.83**	**1.44**	**0.49**	**0.73**	**0.62**	**1.61**
CD (P=0.05)	**1.81**	**3.14**	**1.07**	**1.61**	**1.35**	**3.51**

* Figures in parentheses are per cent increase and decrease over control

Table 16. Influence of green synthesized AgNPs on blackgram inoculated with *M. phaseolina* under glasshouse conditions

Treatments	Shoot		Root		PDI	Seed yield /plant(g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)		
Green synthesized AgNPs at 0.1%	37.62 (29.80)	11.42 (36.25)	7.53 (43.69)	5.62 (72.06)	39.54 (50.26)	1.75 (56.71)
P. fluorescens @ 2.5kg/ha	35.21 (21.71)	10.76 (32.34)	6.56 (35.37)	3.95 (60.25)	43.87 (44.82)	1.52 (51.61)
Carbendazim at 0.1%	30.33 (12.92)	8.50 (14.35)	5.52 (23.19)	1.85 (15.14)	45.79 (42.40)	1.45 (49.15)
Untreated control	26.41	7.28	4.24	2.57	79.50	0.75
SEd	**0.44**	**0.34**	**0.05**	**0.04**	**0.69**	**0.03**
CD (P=0.05)	**0.97**	**0.74**	**0.12**	**0.10**	**1.51**	**0.07**

* Figures in parentheses are per cent increase and decrease over control

Table 17. Effect of green synthesized AgNPs on brinjal inoculated with *Ralstonia solanacearum* under glasshouse conditions

Treatments	Shoot		Root		PDI	Fruit yield / plant
	Height (cm)	Weight (g)	Length (cm)	Weight (g)		
Green synthesized AgNPs at 0.1%	59.33 (21.70)	102.47 (15.29)	19.85 (28.21)	10.25 (29.70)	16.67 (56.01)	120.45 (16.96)
P. fluorescens @ 2.5kg/ha	57.64 (19.41)	98.98 (12.30)	19.10 (25.39)	9.23 (21.90)	33.34 (37.75)	115.68 (13.54)
Streptomycin sulphate at 500 ppm	53.30 (12.85)	95.70 (9.29)	17.13 (16.81)	8.65 (16.70)	41.67 (27.88)	110.56 (9.54)
Untreated control	50.45	86.80	14.25	7.21	53.33	100.01
SEd	**1.76**	**2.10**	**0.58**	**0.31**	**0.69**	**1.92**
CD (P=0.05)	**3.85**	**4.56**	**1.27**	**0.67**	**1.52**	**4.20**

* Figures in parentheses are per cent increase and decrease over control

Table 18. Management of nematode fungal disease complex involving *M. incognita* and *F. oxysporum* f.sp. *lycopersici* under glasshouse conditions

Treatments	Nematode population		Females /g root	Females with egg mass/ g root	Eggs/ egg mass	Gall index	Wilt incidence
	Soil (250 cc)	Root (5 g)					
Green synthesized AgNPs at 0.1%	24.95 (90.12)	15.20 (80.22)	10.32 (62.97)	4.32 (78.99)	115.27 (51.40)	0.2	25.95 (62.03)
P.fluroscens @ 2.5kg/ha	45.00 (82.18)	35.85 (53.36)	15.85 (43.13)	10.51 (48.88)	122.52 (49.97)	2.0	35.25 (48.43)
Carbofuran @ 1Kg a.i./ha + Carbendazim at 0.1%	58.50 (76.83)	45.51 (40.79)	17.30 (37.93)	15.35 (25.34)	143.00 (45.99)	3.0	45.10 (34.02)
Untreated control	252.49	76.86	27.87	20.56	296.20	5.0	68.35
SEd	**0.95**	**0.57**	**0.43**	**0.26**	**3.03**		**0.70**
CD (P=0.05)	**2.06**	**1.25**	**0.94**	**0.58**	**6.61**		**1.53**

* Figures in parentheses are per cent decrease over control

Table 19. Influence of green synthesized AgNPs on plant growth characters of tomato infected with *M. incognita* and *F. oxysporum* f.sp. *lycopersici* under glasshouse conditions

Treatments	Shoot		Root		Fruit yield /plant(g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.1%	67.44 (21.19)	110.34 (38.87)	32.16 (26.00)	27.36 (34.21)	97.51 (13.47)
P. fluorescens @ 2.5kg/ha	65.65 (19.04)	106.84 (36.87)	30.94 (23.07)	25.53 (29.49)	95.45 (11.60)
Carbofuran @ 1Kg a.i./ha + Carbendazim at 0.1%	61.31 (13.31)	98.70 (31.66)	27.12 (12.23)	21.29 (15.45)	92.09 (8.37)
Untreated control	53.15	67.45	23.80	18.00	84.38
SEd	**1.34**	**1.29**	**0.98**	**0.47**	**1.67**
CD (P=0.05)	**2.91**	**2.83**	**2.13**	**1.02**	**3.59**

* Figures in parentheses are per cent increase over control

Table 20. Management of nematode fungal disease complex involving *M. incognita* and *M. phaesolina* in blackgram

Treatments	Nematode population		Females /g root	Females with egg mass/ g root	Eggs/ egg mass	Gall index	Wilt incidence
	Soil (250 cc)	Root (5 g)					
Green synthesized AgNPs at 0.1%	37.32 (85.29)	27.50 (54.07)	4.01 (80.06)	3.91 (73.59)	124.71 (34.98)	0.4	31.87 (53.81)
P. fluorescens @ 2.5 kg/ha	56.85 (77.59)	33.12 (44.68)	7.53 (62.57)	5.88 (60.30)	137.97 (28.07)	1.0	38.20 (44.64)
Carbofuran @1Kg a.i./ha + Carbendazim at 0.1%	61.25 (75.85)	37.31 (37.68)	11.44 (43.14)	7.19 (51.45)	154.66 (19.37)	2.0	44.61 (35.35)
Untreated control	253.63	59.87	20.12	14.81	191.81	5.0	69.00
SEd	**1.43**	**1.19**	**0.17**	**0.14**	**0.72**		**1.28**
CD (P=0.05)	**3.13**	**2.60**	**0.38**	**0.32**	**1.57**		**2.79**

* Figures in parentheses are per cent decrease over control

Table 21. Influence of green synthesized AgNPs on plant growth characters of blackgram infected by *M. incognita* and *M. phaesolina* under glasshouse conditions

Treatments	Shoot		Root		Seed yield /plant (g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.1%	57.50 (23.39)	96.48 (10.34)	33.25 (28.20)	31.06 (33.81)	2.00 (47.61)
P. fluorescens @ 2.5 kg/ha	55.44 (20.54)	93.86 (7.84)	31.41 (23.99)	27.40 (24.96)	1.85 (43.37)
Carbofuran @1Kg a.i./ha + Carbendazim at 0.1%	50.38 (12.56)	89.56 (3.42)	27.49 (13.13)	23.65 (13.07)	1.42 (26.34)
Untreated control	44.05	86.50	23.88	20.56	1.05
SEd	**1.31**	**1.01**	**1.08**	**0.96**	**0.04**
CD (P=0.05)	**2.87**	**2.20**	**2.36**	**2.08**	**0.09**

* Figures in parentheses are per cent increase over control

Table 22. Management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* in brinjal under glasshouse conditions

Treatments	Nematode population		Females /g root	Females with egg mass/ g root	Eggs/ egg mass	Gall index	Wilt incidence
	Soil (250 cc)	Root (5g)					
Green synthesized AgNPs at 0.1%	45.11 (82.75)	25.68 (68.12)	6.00 (62.05)	2.40 (71.12)	106.86 (62.06)	0.6	21.91 (57.26)
P. fluorescens @ 2.5 kg/ha	66.00 (74.76)	30.87 (60.75)	10.25 (35.17)	4.12 (50.42)	124.26 (55.88)	1	28.21 (44.96)
Carbofuran @ 1Kg a.i./ha+ Streptomycin sulphate at 500 ppm	74.50 (71.51)	42.99 (46.33)	13.37 (15.43)	6.60 (20.58)	160.19 (43.12)	2	33.49 (34.65)
Untreated control	261.45	80.56	15.81	8.31	281.62	5	51.25
SEd	**1.90**	**1.84**	**0.20**	**0.08**	**2.21**		**0.96**
CD (P=0.05)	**4.13**	**2.58**	**0.44**	**0.18**	**4.83**		**2.10**

* Figures in parentheses are per cent decrease over control

Table 23. Influence of green synthesized AgNPs on plant growth characters of brinjal infected with *M. incognita* and *R. solanacearum* under glasshouse conditions

Treatments	Shoot		Root		Fruit yield /plant (g)
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.1%	75.68 (18.16)	130.90 (7.39)	42.90 (29.14)	25.85 (28.86)	110.45 (18.51)
P. fluorescens @ 2.5kg/ha	71.22 (13.03)	129.11 (6.11)	37.80 (19.58)	24.51 (24.97)	105.68 (14.83)
Carbofuran @ 1Kg a.i./ha+ Streptomycin sulphate 500 ppm	67.32 (7.99)	125.55 (3.45)	33.92 (10.38)	22.18 (17.09)	100.56 (10.49)
Untreated control	61.94	121.22	30.40	18.39	90.01
SEd	**0.82**	**2.27**	**1.46**	**0.81**	**1.16**
CD (P=0.05)	**1.79**	**4.95**	**3.10**	**1.78**	**2.53**

* Figures in parentheses are per cent increase over control.

Table 24. Influence of green synthesized AgNPs on *M. incognita* in tomato in plant growth chamber

Treatments	Nematode population		Females/ g root	Females with egg mass/g root	Eggs/ egg mass	Gall index
	Soil (250cc)	Root (5g)				
Green synthesized AgNPs at 0.0005%	14.00 (72.00)	4.00 (87.09)	2.00 (71.43)	1.00 (80.00)	124.00 (36.73)	0 (100.00)
Untreated control	50.00	31.00	7.00	5.00	206.00	2

* Figures in parentheses are per cent decrease over control.

Table 25. Influence of green synthesized AgNPs on plant growth characters of tomato challenged with *M. incognita* in plant growth chamber

Treatments	Shoot		Root	
	Height(cm)	Weight (g)	Length (cm)	Weight (g)
Green synthesized AgNPs at 0.0005%	17.50 (5.14)	28.45 (10.37)	12.30 (34.95)	9.65 (58.54)
Untreated control	16.60	25.50	8.00	4.00

* Figures in parentheses are per cent increase over control.

Table 26. Influence of green synthesized AgNPs on tomato inoculated with *F. oxysporum* f.sp. *lycopercsici* in plant growth chamber

Treatments	Shoot		Root		Wilt incidence
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.1%	18.50 (10.81)	25.00 (2.60)	13.00 (19.23)	8.50 (41.18)	0 (100.00)
Untreated control	16.50	24.35	10.50	5.00	2

* Figures in parentheses are per cent increase and decrease over control.

Table 27. Influence of green synthesized AgNPs on blackgram inoculated with *M. phaseolina* in plant growth chamber

Treatments	Shoot		Root		Wilt incidence
	Height(cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0. 1%	15.30 (5.22)	20.15 (3.22)	8.50 (5.88)	5.35 (6.54)	0 (100.00)
Untreated control	14.50	19.50	8.00	5.00	2

* Figures in parentheses are per cent increase and decrease over control.

Table 28. Effect of green synthesized AgNPs on brinjal inoculated with *R. solanacearum* in plant growth chamber

Treatments	Shoot		Root		Wilt incidence
	Height (cm)	Weight (g)	Length (cm)	Weight (g)	
Green synthesized AgNPs at 0.1%	17.50 (8.57)	10.00 (5.00)	5.50 (8.33)	7.50 (0.53)	0 (100.00)
Untreated control	16.00	9.50	6.00	7.54	2

* Figures in parentheses are per cent increase and decrease over control.

Table 29. Management of nematode fungal disease complex involving *M. incognita* and *F. oxysporum* f.sp. *lycopersici* in plant growth chamber

Treatments	Nematode population		Females /g root	Females with egg mass/ g root	Eggs/ egg mass	Gall index	Wilt incidence
	Soil (250cc)	Root (5 g)					
Green synthesized AgNPs at 0.1%	26.60 (58.43)	10.00 (64.29)	2.00 (66.67)	1.00 (80.00)	142.00 (24.86)	0.00 (100.00)	0 (100.00)
Untreated control	64.00	28.00	6.00	5.00	189.00	1.00	1

* Figures in parentheses are per cent decrease over control.

Table 30. Influence of green synthesized AgNPs on plant growth characters of tomato infected with *M. incognita* and *F. oxysporum* f.sp. *lycopersici* in plant growth chamber

Treatments	Shoot		Root	
	Height(cm)	Weight (g)	Length (cm)	Weight (g)
Green synthesized AgNPs at 0.1%	15.00 (10.00)	24.53 (16.80)	10.00 (35.00)	8.85 (37.85)
Untreated control	13.50	20.41	06.50	5.50

* Figures in parentheses are per cent increase over control.

Table 31. Influence of green synthesized AgNPs on nematode fungal disease complex of blackgram involving *M. incognita* and *M. phaesolina* in plant growth chamber

Treatments	Nematode population		Females /g root	Females with egg mass/g root	Eggs/ egg mass	No of galls/plant	Wilt incidence
	Soil (250 cc)	Root (5 g)					
Green synthesized AgNPs at 0.1%	06.00 (89.83)	3.00 (80.00)	2.00 (71.43)	1.00 (80.00)	154.00 (22.22)	0.00 (100.00)	0 (100.00)
Untreated control	59.00	15.00	7.00	5.00	198.00	21.00	2

* Figures in parentheses are per cent decrease over control.

Table 32. Influence of green synthesized AgNPs on plant growth characters of blackgram infected with *M. incognita* and *M. phaesolina* in plant growth chamber

Treatments	Shoot		Root	
	Height(cm)	Weight (g)	Length (cm)	Weight (g)
Green synthesized AgNPs at 0.1%	18.50 (18.92)	23.55 (17.49)	13.00 (3.84)	9.53 (31.79)
Untreated control	15.00	19.43	12.50	6.50

* Figures in parentheses are per cent increase over control.

Table 33. Influence of green synthesized AgNPs on nematode bacterial disease complex of brinjal involving *M. incognita* and *R. solanacearum* in plant growth chamber

Treatments	Nematode population		Females /g root	Females with egg mass/ g root	Eggs/ egg mass	Gall index	Wilt incidence
	Soil (250 cc)	Root (5g)					
Green synthesized AgNPs at 0.1%	10.00 (89.58)	5.00 (80.78)	2.00 (81.82)	1.00 (87.5)	160.00 (17.10)	0 (100.00)	0 (100.00)
Untreated control	96.00	26.00	11.00	8.00	193.00	2	2

* Figures in parentheses are per cent decrease over control.

Table 34. Influence of green synthesized AgNPs on plant growth characters of brinjal Infected with *M. incognita* and *R. solanacearum* in plant growth chamber

Treatments	Shoot		Root	
	Height(cm)	Weight (g)	Length (cm)	Weight (g)
Green synthesized AgNPs at 0.1%	25.00 (18.40)	15.00 (9.87)	11.00 (13.64)	8.50 (20.58)
Untreated control	20.40	13.52	09.50	6.75

* Figures in parentheses are per cent increase over control.

Table 35. Effect of green synthesized AgNPs on juveniles of *Steinernema glaseri*

Concentrations of AgNPs	Mortality of juveniles (%) at different intervals (Days)				
	1	**2**	**3**	**4**	**5**
0.0005 %	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
0.0001%	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
0.00005 %	26.89 (31.24)	53.78 (47.17)	79.56 (63.12)	100.00 (90.00)	100.00 (90.00)
0.00001%	50.40 (45.23)	60.50 (51.06)	64.97 (53.71)	75.70 (60.47)	82.30 (65.12)
Untreated control	1.00 (5.74)	2.00 (8.13)	5.57 (13.65)	7.41 (15.80)	9.56 (18.01)
SEd	**0.27**	**0.55**	**0.29**	**0.37**	**0.58**
C.D(P=0.05)	**0.62**	**1.22**	**0.65**	**0.80**	**1.29**

*Figures in parentheses are arcsine transformed values.

Table 36. Effect of green synthesized AgNPs on beneficial fungus *Trichoderma viride*

Concentrations of AgNPs (%)	Radial growth of the fungus (mm)	Decrease over control (%)
0.010	67.50	22.81
0.025	60.00	29.06
0.050	42.50	43.15
0.075	36.50	48.06
0.100	31.50	52.29
Untreated control	90.00	0.00
SEd	**0.47**	-
CD(P=0.05)	**1.03**	-

Table 37. Effect of green synthesized AgNPs on nematode egg parasitic fungus *Paecilomyces lilacinus*

Concentrations of AgNPs (%)	Radial growth of the pathogen (mm)	Decrease over control (%)
0.010	65.30	27.44
0.025	60.50	32.78
0.050	56.20	37.56
0.075	52.60	41.56
0.100	45.80	49.11
Untreated control	90.00	0.00
SEd	**1.41**	**-**
CD(P=0.05)	**3.09**	**-**

Table 38. Effect of green synthesized AgNPs on nematode bacterial bioagents

Concentrations of AgNPs (%)	Zone of inhibition (mm)	
	Pseudomonas fluorescens	***Bacillus subtilis***
0.025	12.83 (96.10)	12.15 (95.88)
0.050	14.92 (96.65)	14.88 (96.64)
0.075	16.42 (96.95)	15.42 (96.76)
0.100	17.58 (97.16)	17.13 (97.08)
Untreated control	0.50	0.50
SEd	**0.05**	**0.03**
CD(P=0.05)	**0.12**	**0.07**

* Figures in parentheses are per cent increase over control.

Figure 17. Effect of green synthesized AgNPs *in vitro* on egg hatchability of *M. incognita*

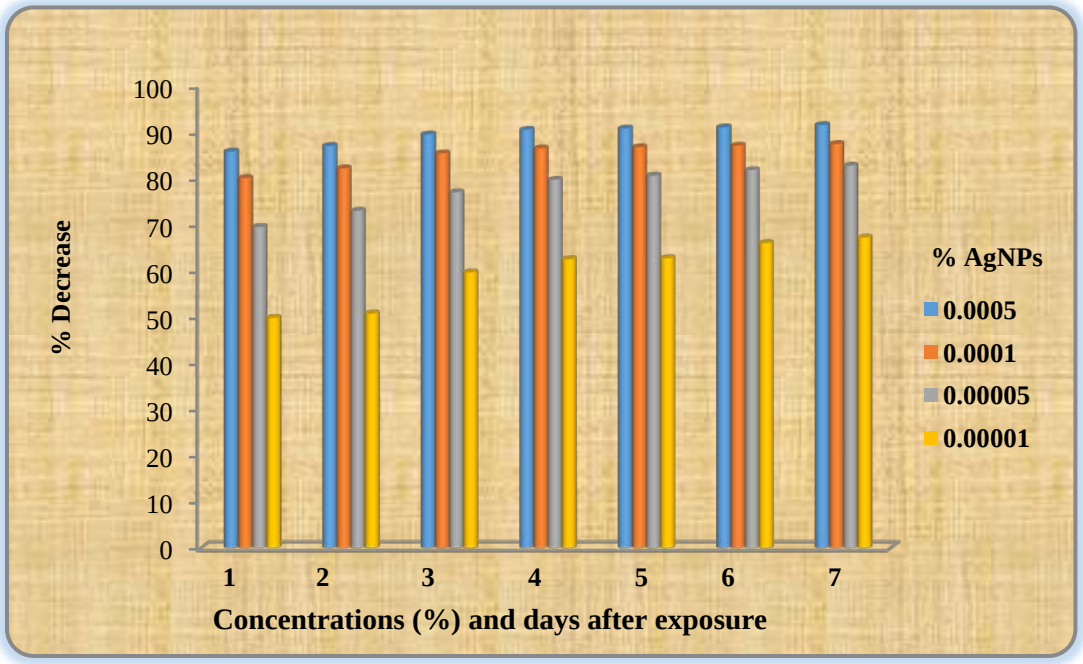


Figure 18. Effect of green synthesized AgNPs *in vitro* on juveniles of *M. incognita*

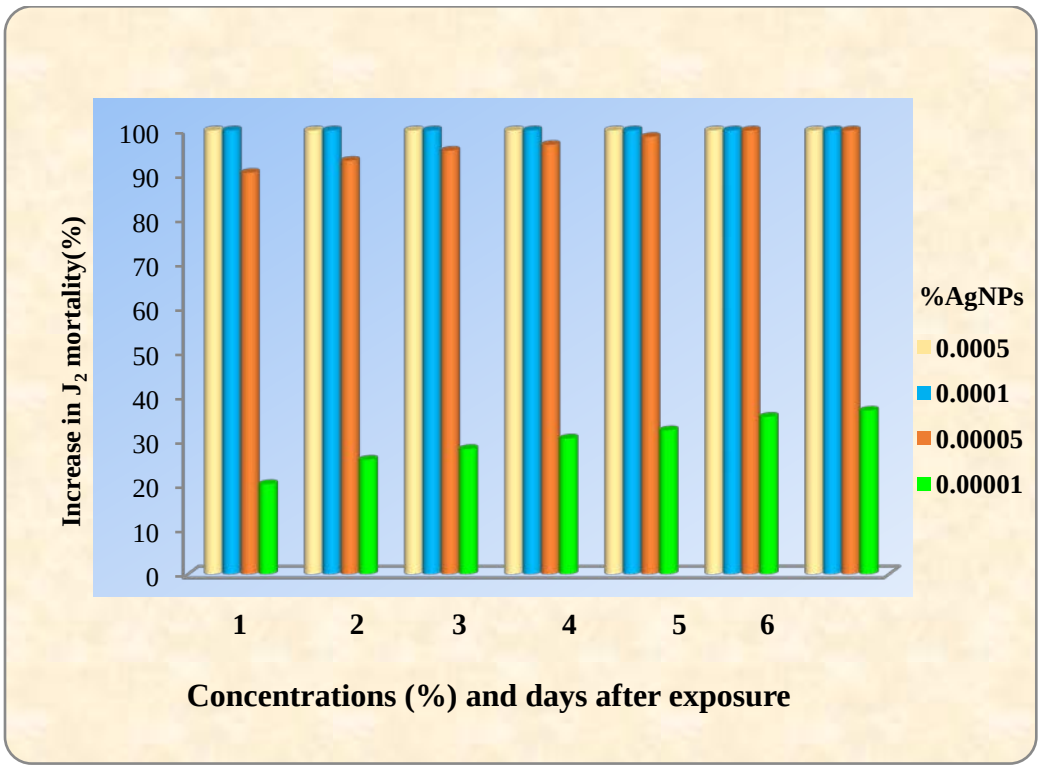


Figure 19. Effect of green synthesized AgNPs on attraction of *M. incognita* juveniles towards roots of tomato *in vitro*

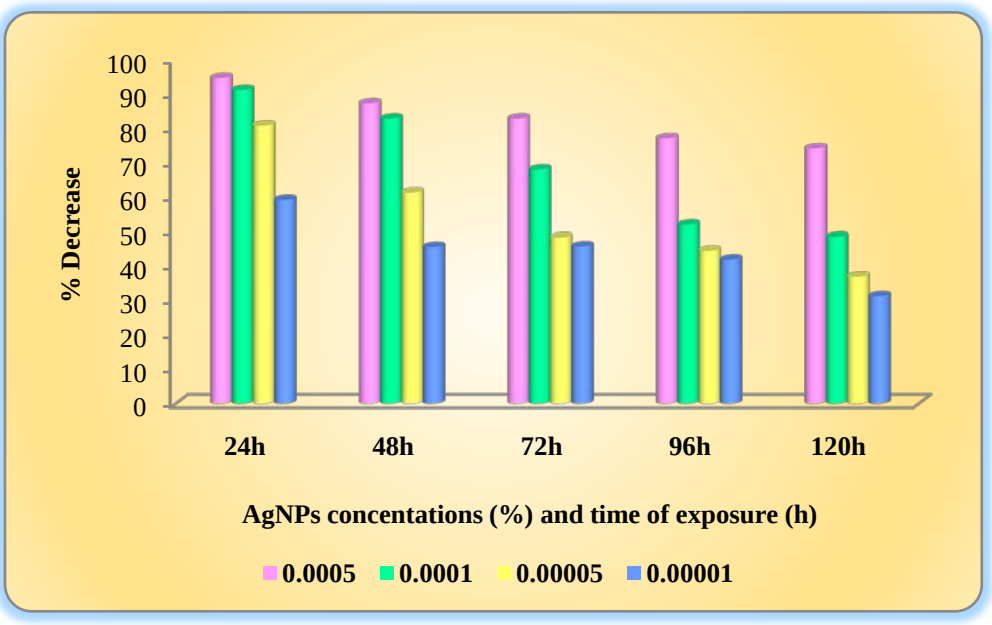


Figure 20. Effect of green synthesized AgNPs on root penetration of *M. incognita* juveniles in tomato

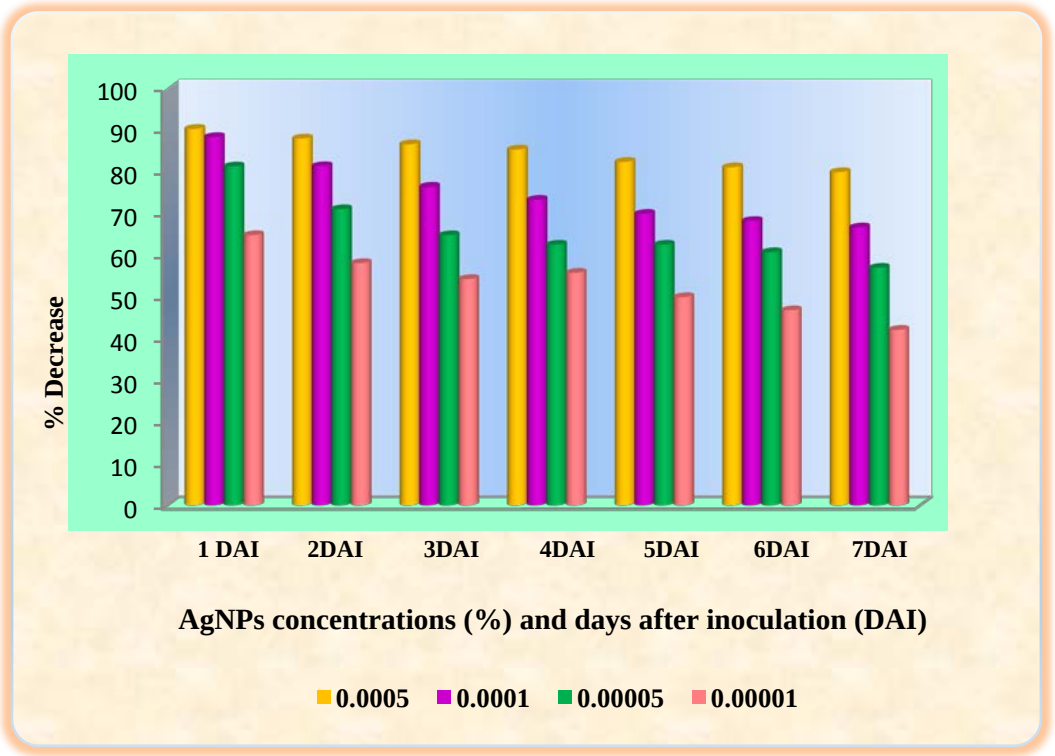


Figure 21. Effect of green synthesized AgNPs *in vitro* on mycelial growth of *F. oxysporum* f.sp. *lycopersici*

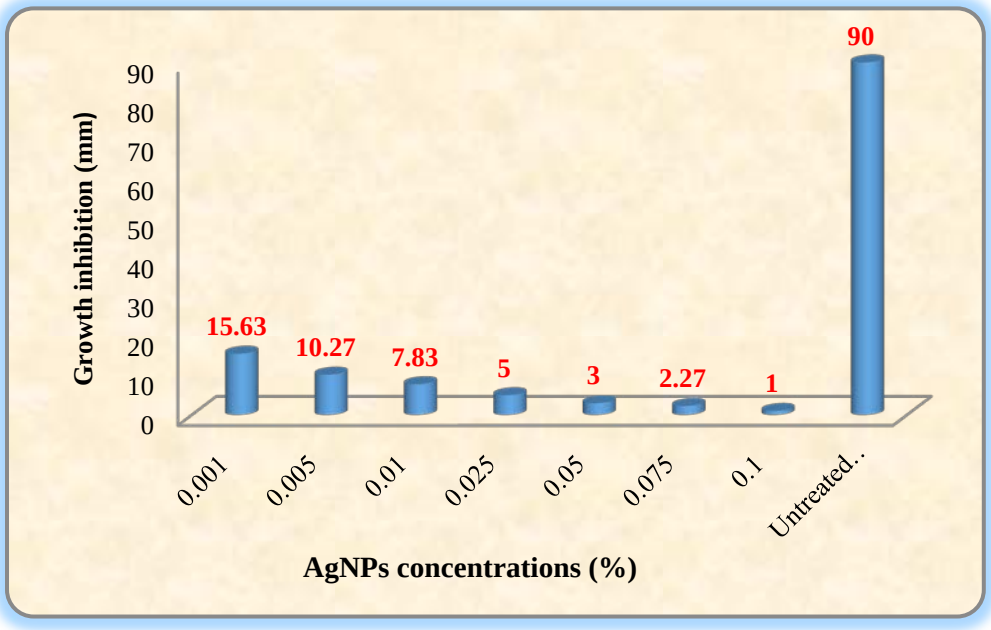


Figure 22. Effect of green synthesized AgNPs *in vitro* on mycelial growth of *M. phaseolina*

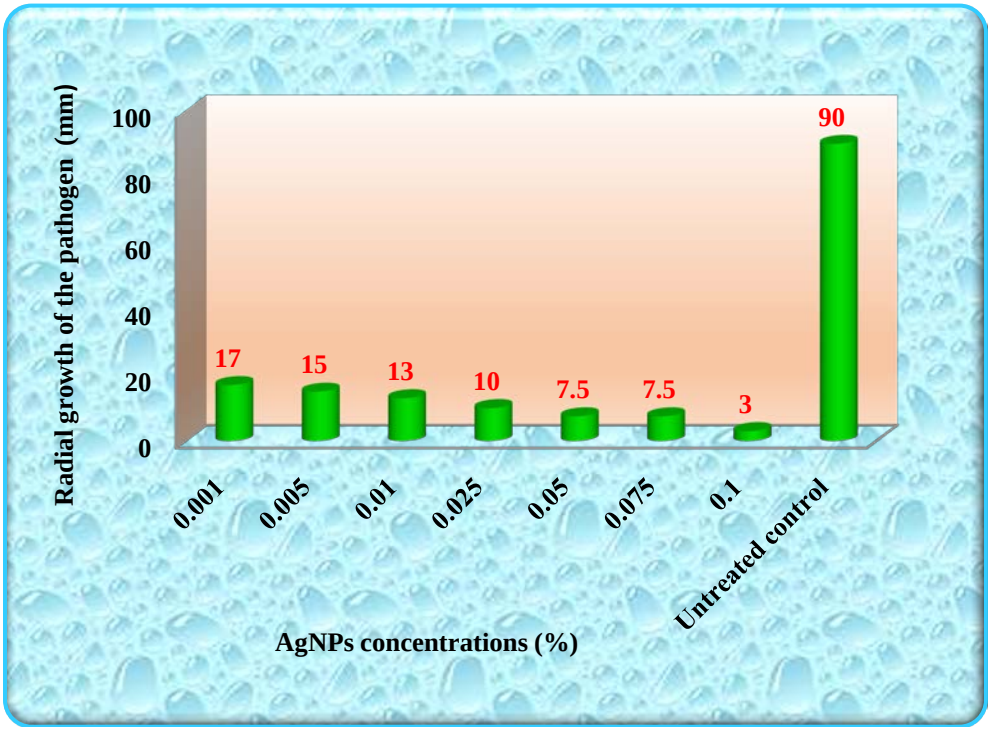


Figure 23. ***In vitro*** **effect of green synthesized AgNPs on bacterial pathogen of** ***R. solanacearum***

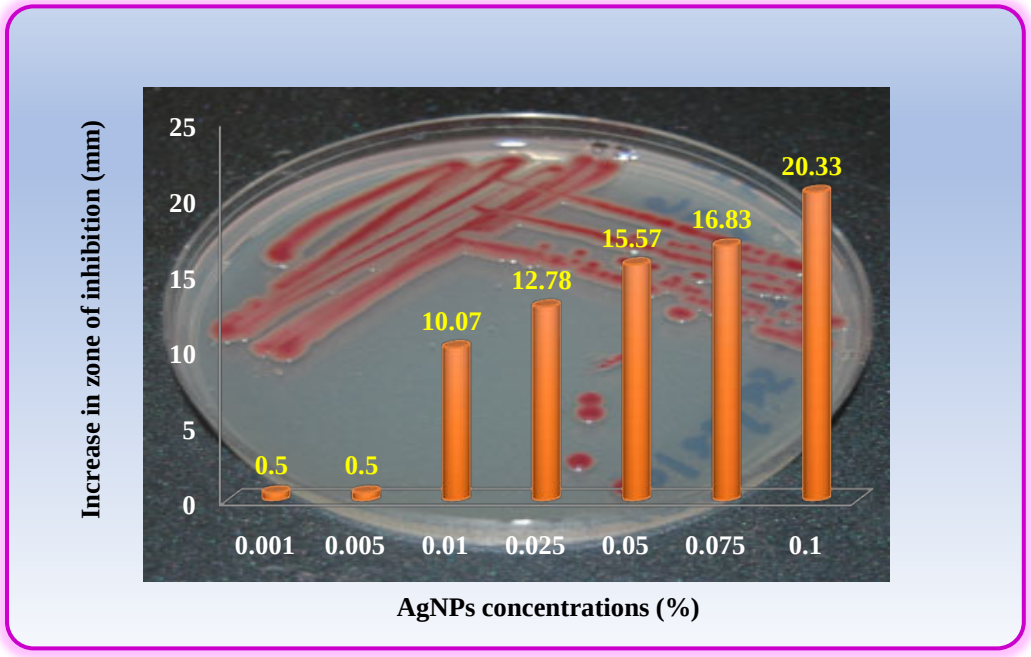


Figure 24. ***In vitro*** **effect of green synthesized AgNPs on bacterial pathogen of** ***X. oryzae*** **pv.** ***oryzae***

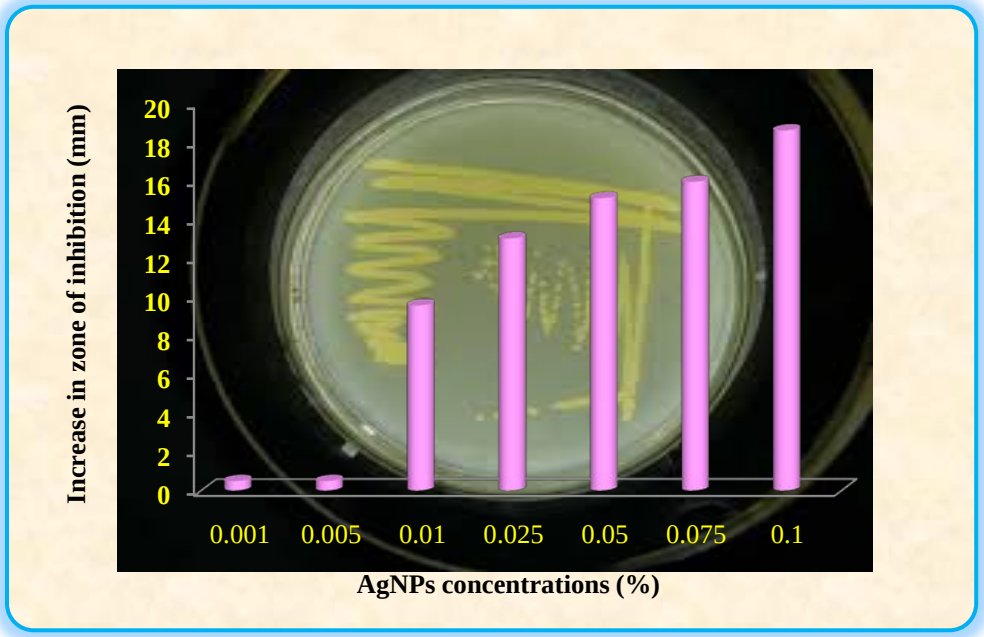


Figure 25. Influence of green synthesized AgNPson *M. incognita* in tomato under glasshouse conditions

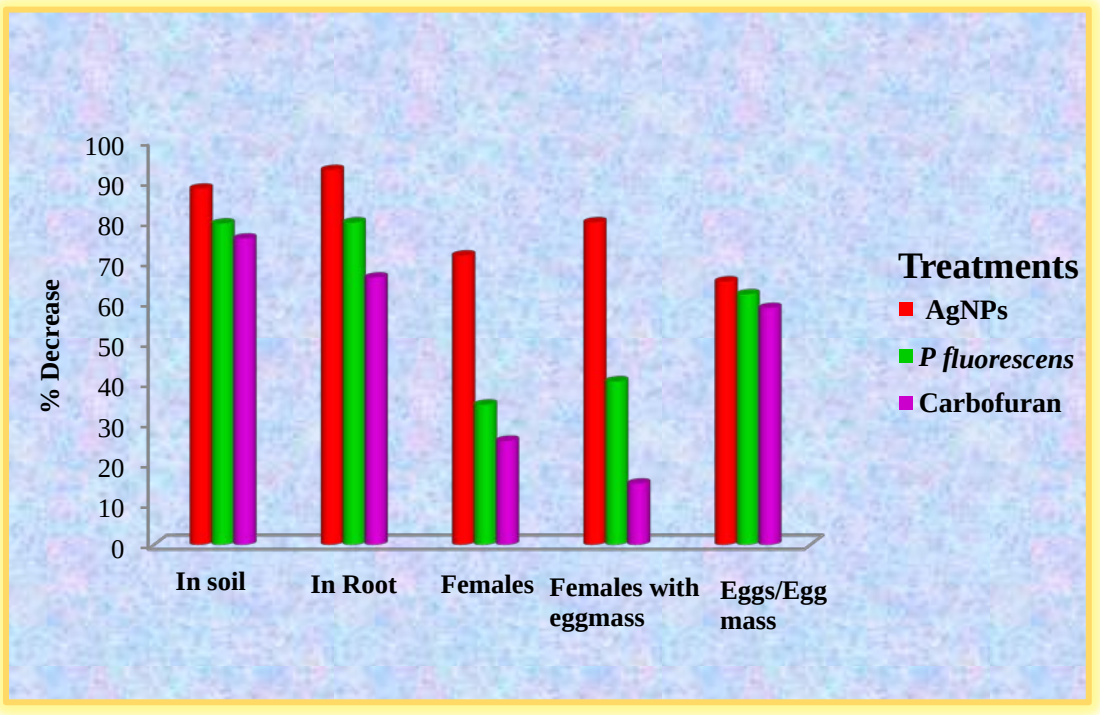


Figure 26. Influence of green synthesized AgNPson plant growth characters of tomato challenged with *M. incognita* under glasshouse conditions

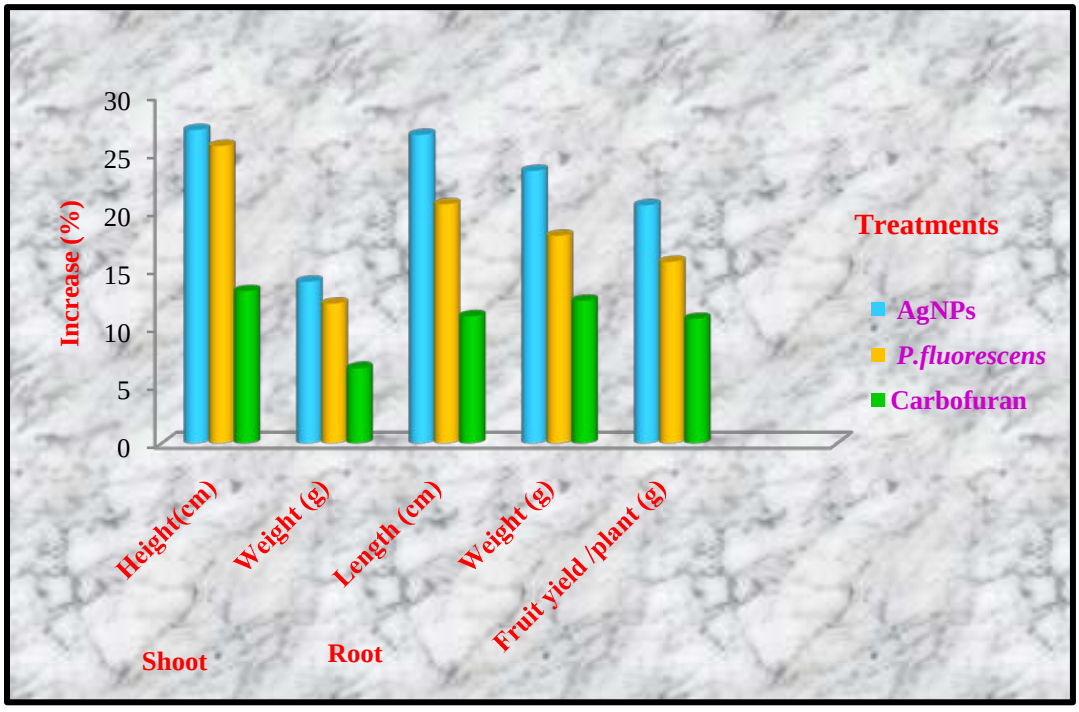


Figure 27. Influence of green synthesized AgNPs on plant growth of tomato inoculated with *F. oxysporum* f.sp. *lycopercsici* under glasshouse conditions

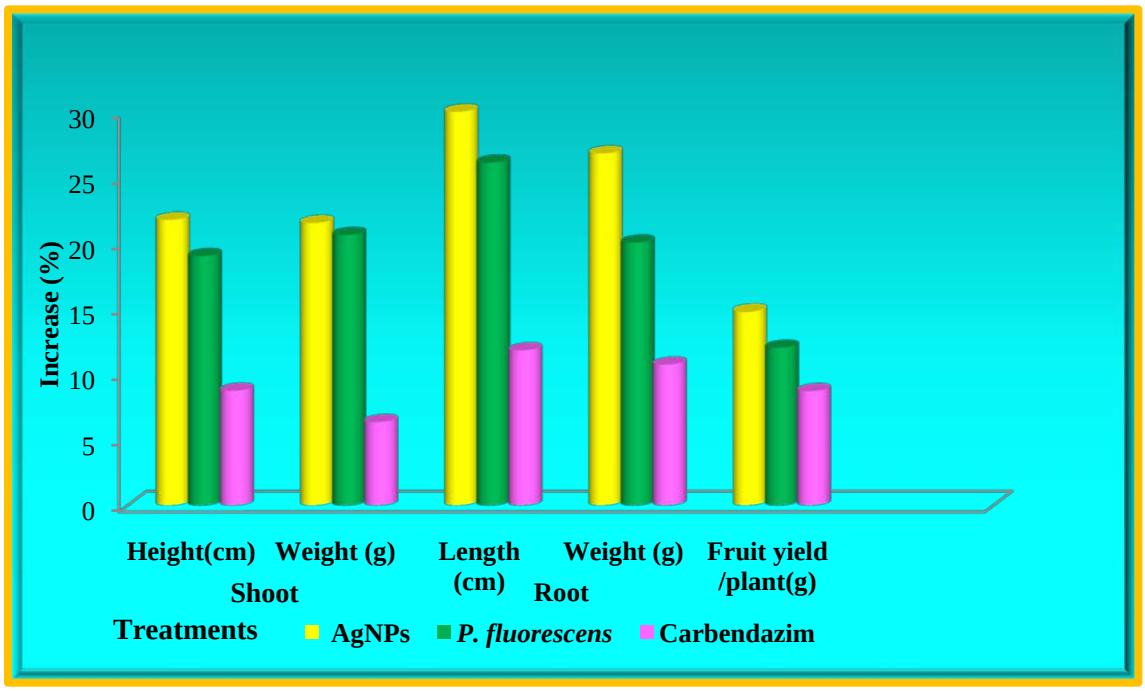


Figure 28. Influence of green synthesized AgNPs on plant growth of blackgram inoculated with *M. phaseolina* under glasshouse conditions

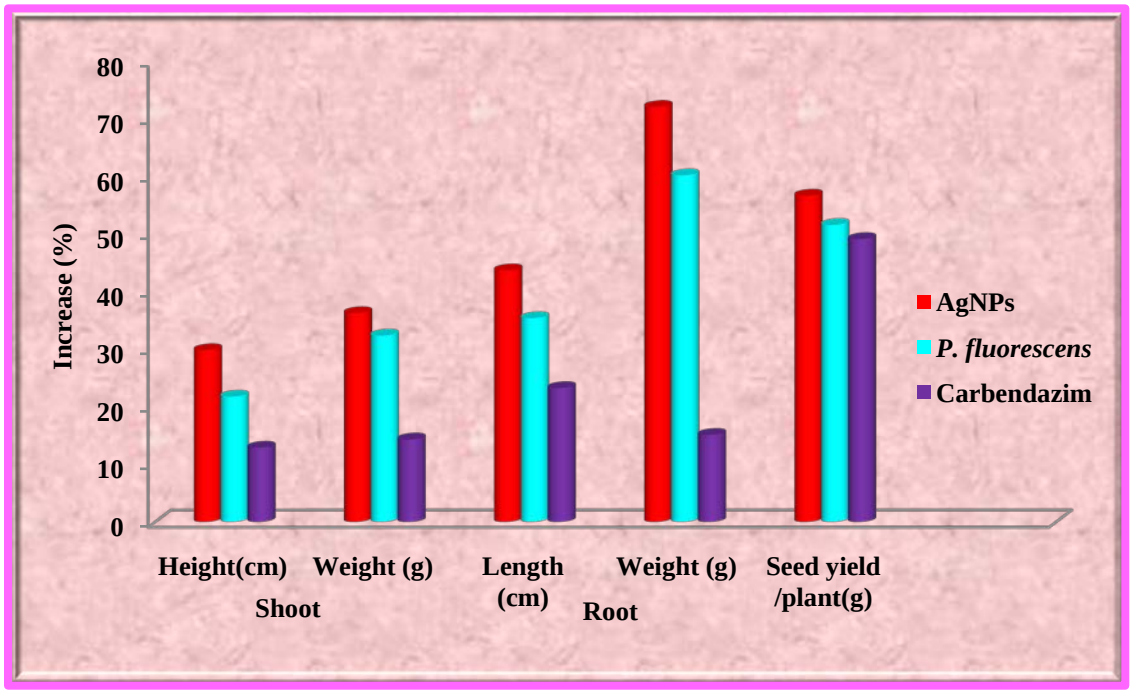


Figure 29. Effect of green synthesized AgNPs on plant growth brinjal inoculated with *R. solanacearum* under glasshouse conditions

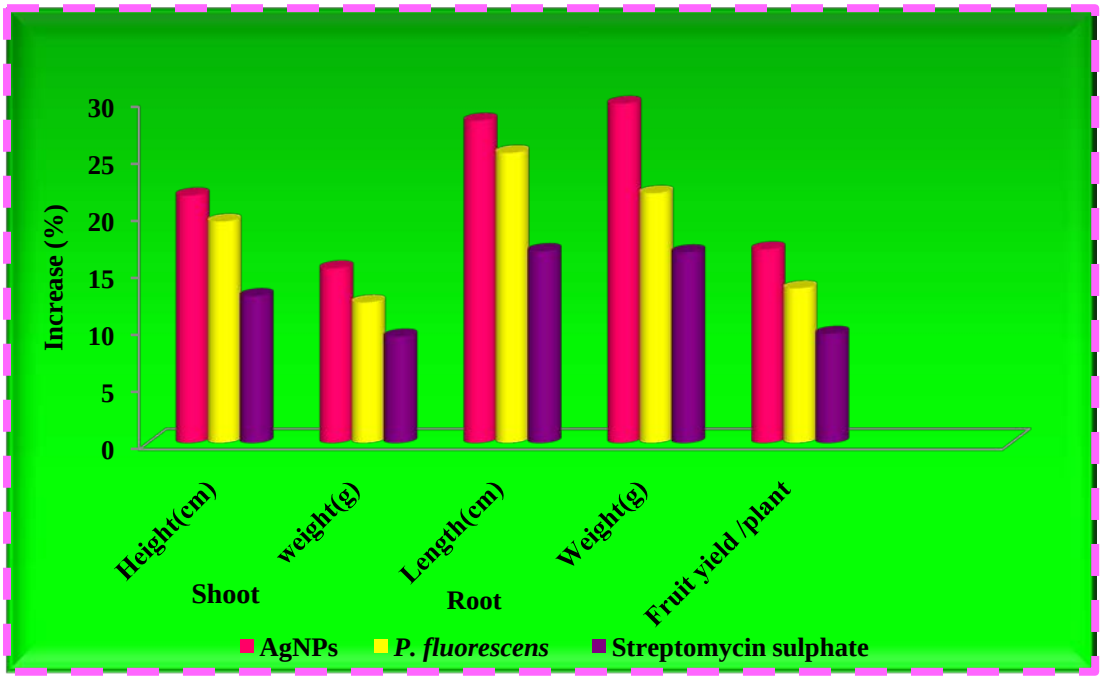


Figure 30. Management of nematode fungal disease complex involving *M. incognita* and *F. oxysporum* f.sp. *lycopersici* under glasshouse conditions

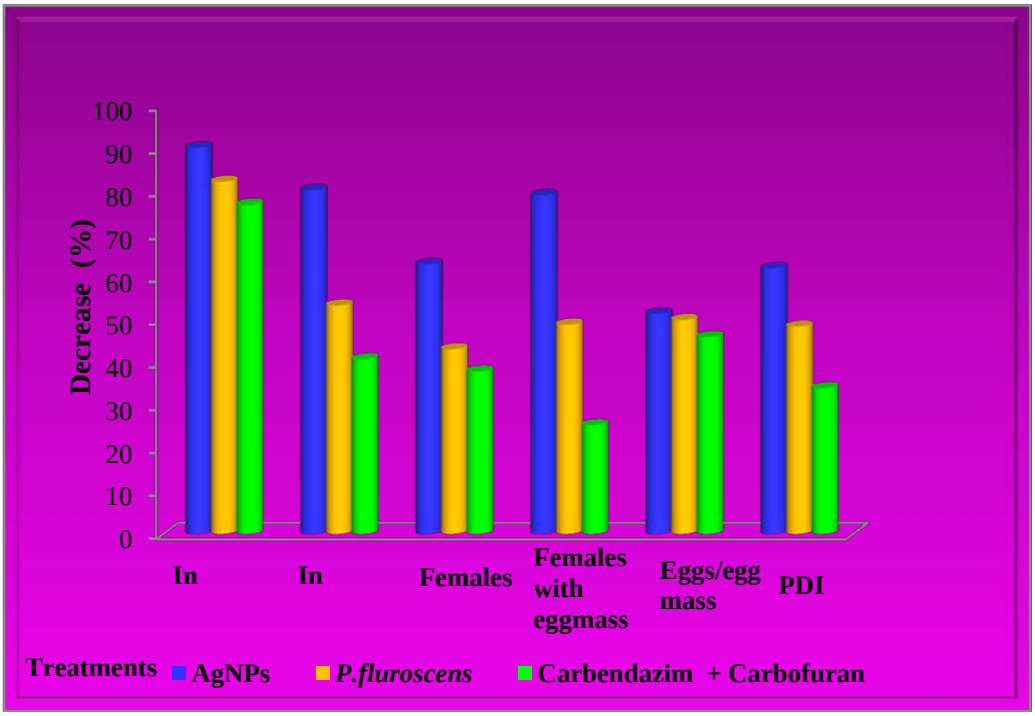


Figure 31. Influence of green synthesized AgNPson plant growth characters of tomato infected with *M. incognita* and *F. oxysporum* f.sp. *lycopersici* under glasshouse conditions

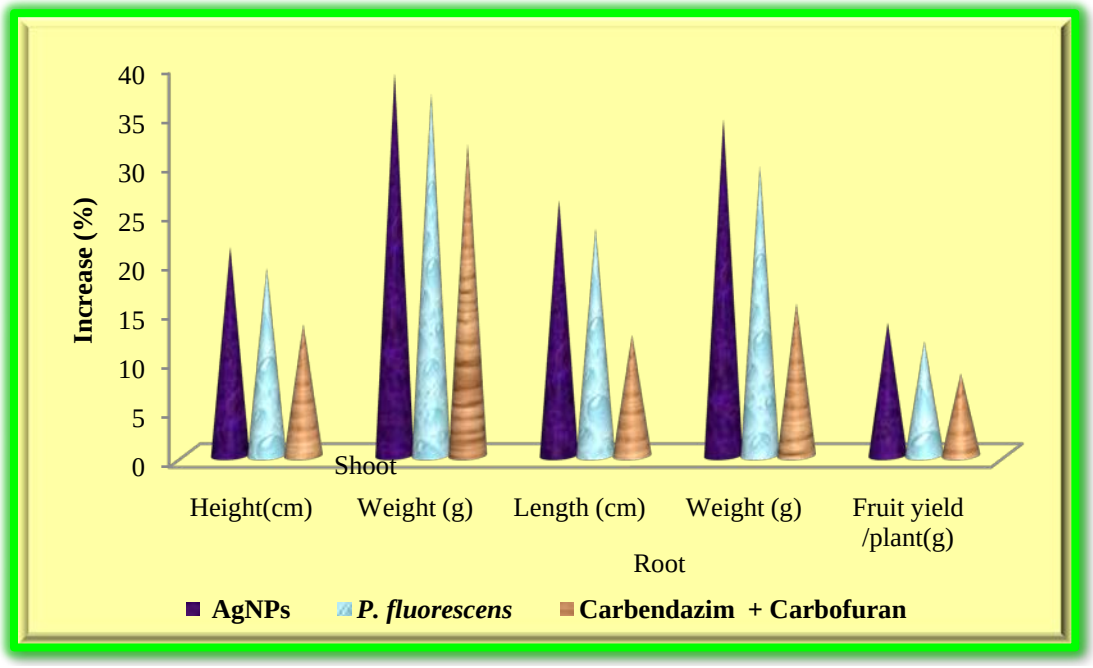


Figure 32. Management of nematode fungal disease complex involving *M. incognita* and *M. phaesolina* in blackgram

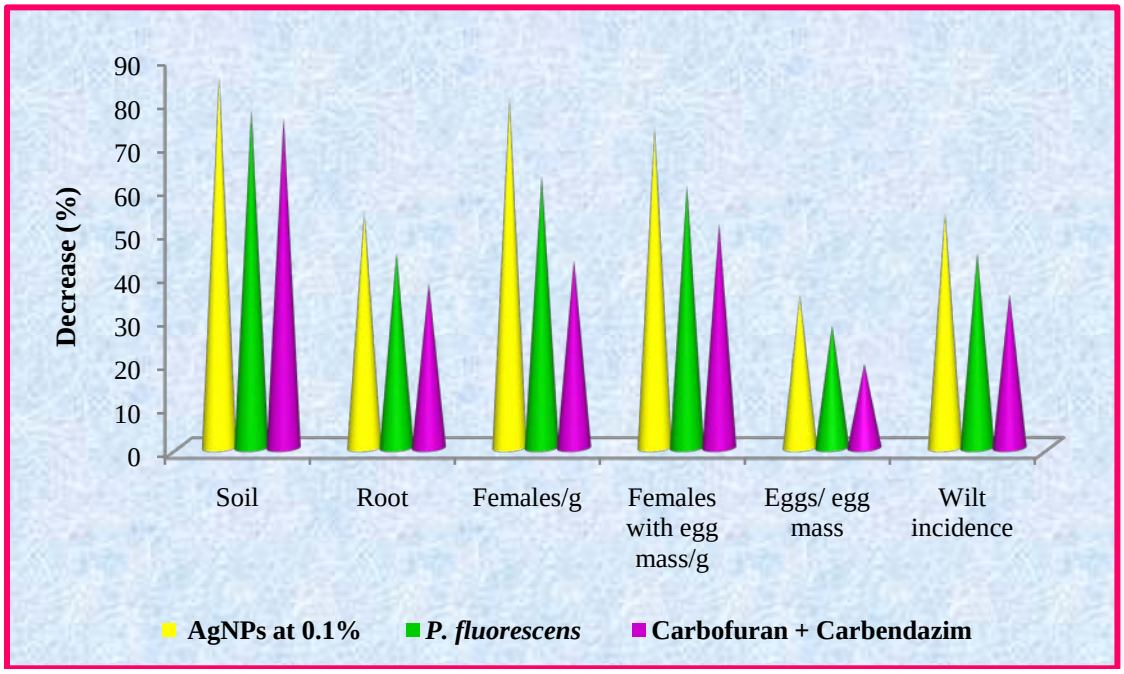


Figure 33. Influence of green synthesized AgNPs on plant growth characters of blackgram infected by *M. incognita* and *M. phaesolina* under glasshouse conditions

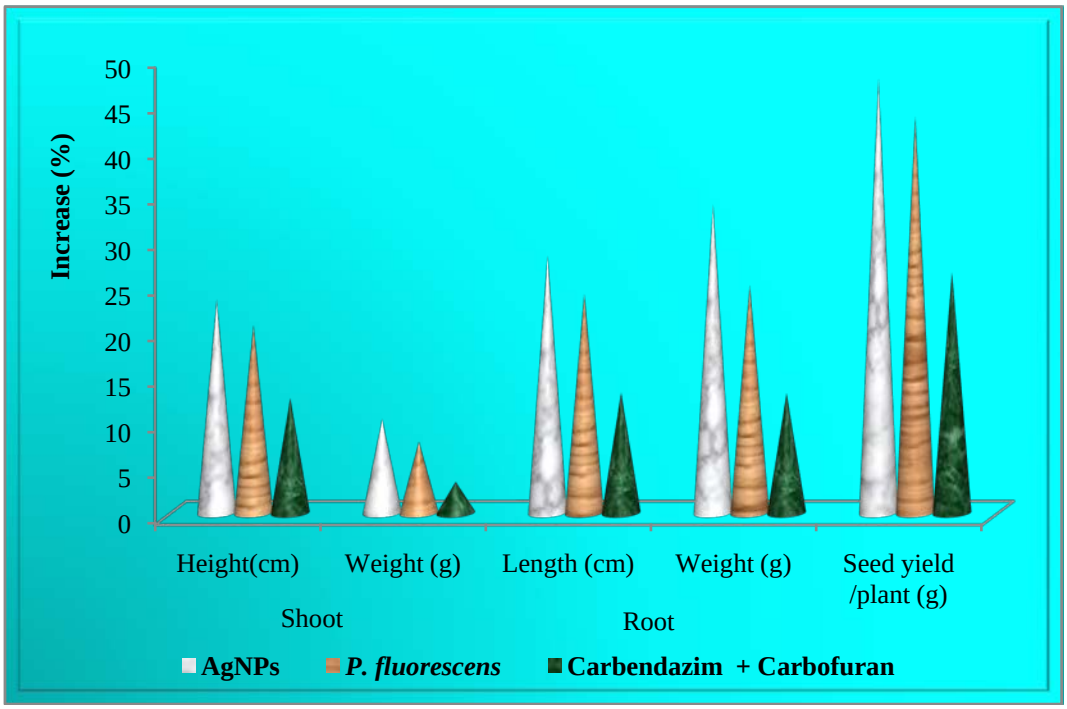


Figure 34. Management of nematode bacterial disease complex involving *M. incognita* and *R. solanacearum* in brinjal under glasshouse conditions

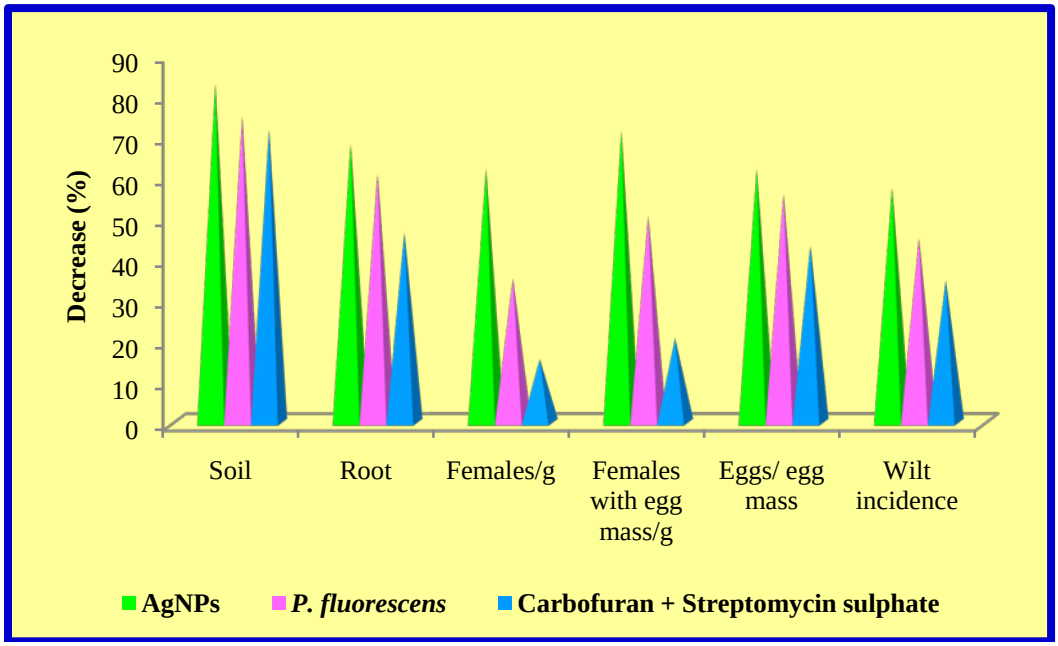


Figure 35. Influence of green synthesized AgNPs on plant growth characters of brinjal infected with *M. incognita* and *R. solanacearum* under glasshouse conditions

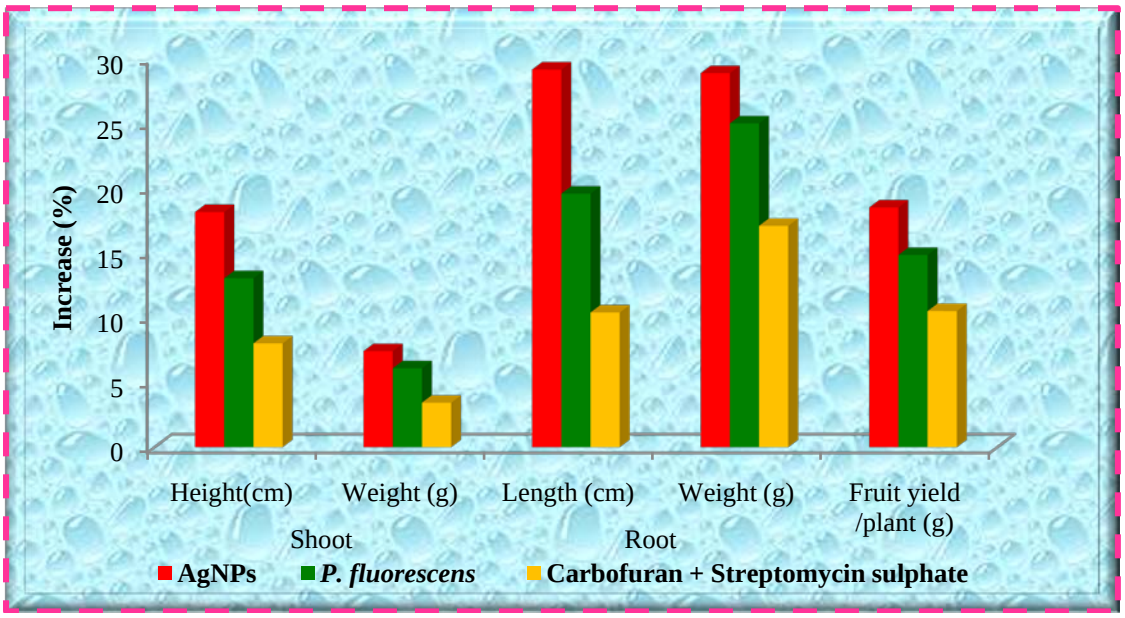


Figure 36. Effect of green synthesized AgNPs on juveniles of *Steinernema glaseri*

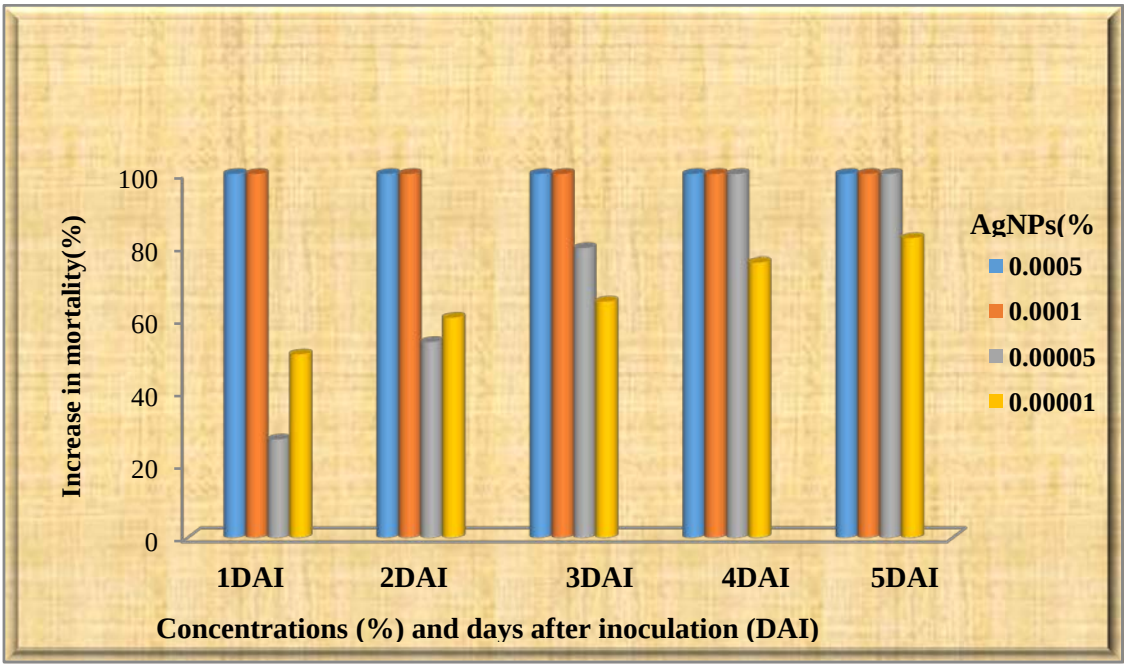


Figure 37. Effect of green synthesized AgNPs on beneficial fungi

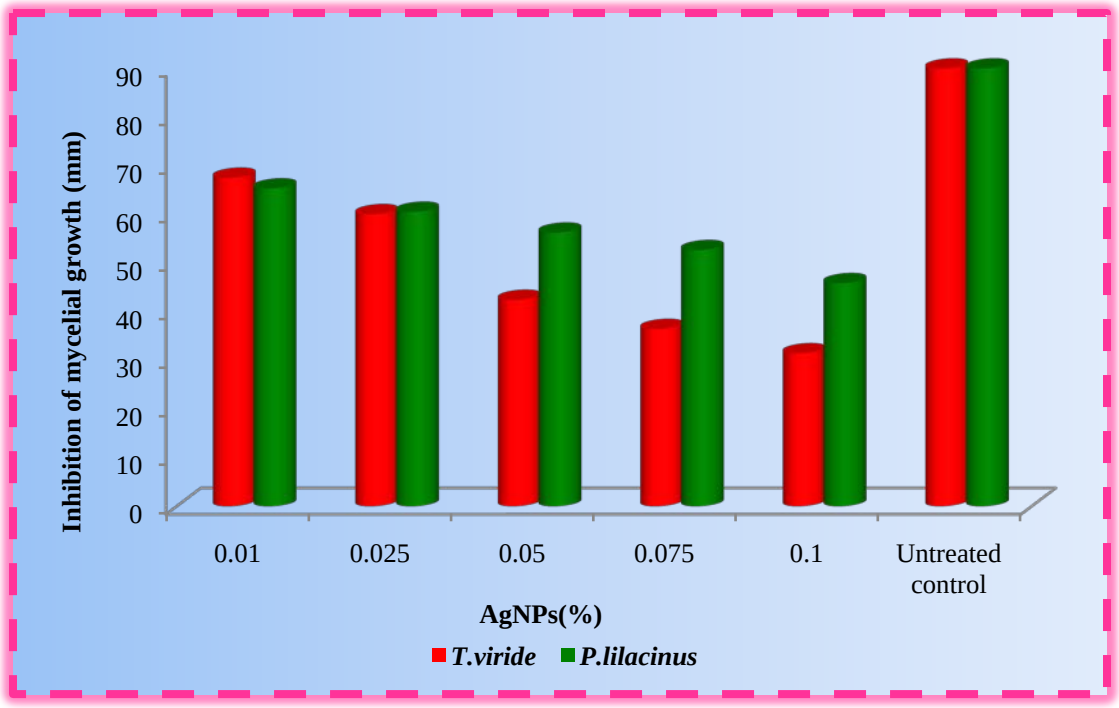


Figure 38. Effect of green synthesized AgNPs on nematode bacterial bioagents

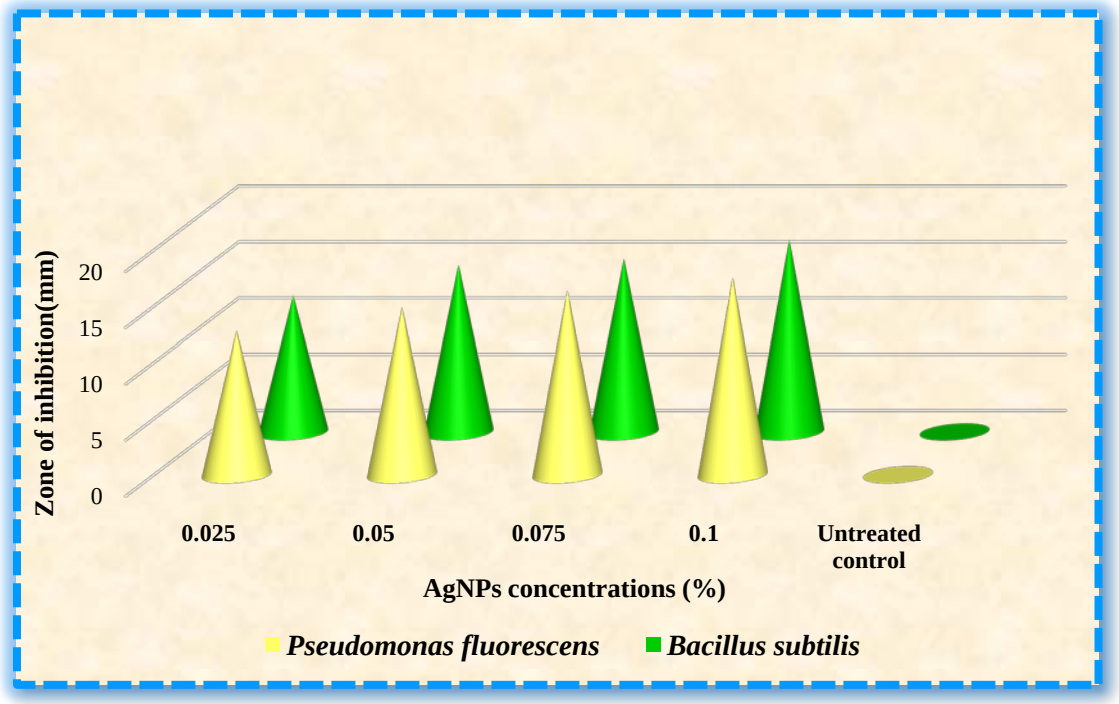


Figure 1. Flow chart for synthesis of nanoparticles

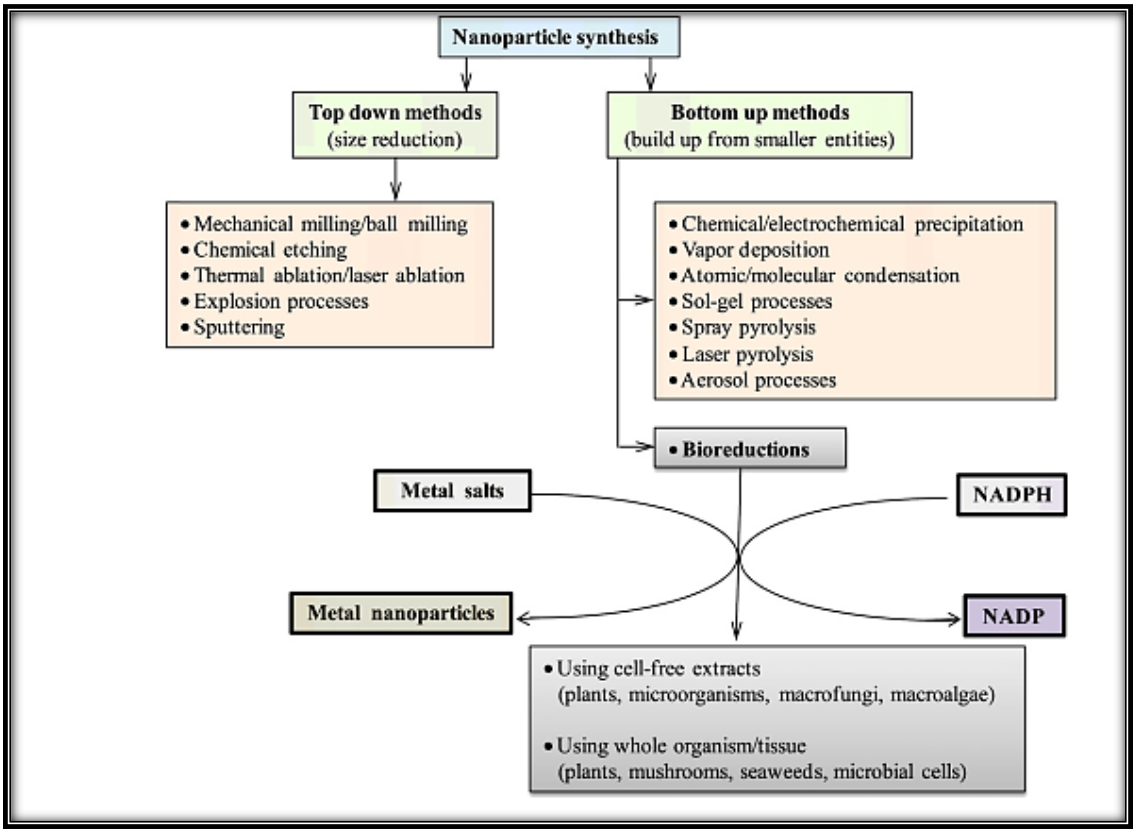


Plate 1. Flowchart explaining green synthesis of AgNPs

Step 1. Boiling of bits of leaves (90^{o}C) and filtrating the extracts

Step 2: Boiling (90^{o}C) silver nitrate at 0.01M for 15 min

Step 3: Adding leaf extracts to boiled $AgNO_3$

Step 4: Centrifuging at 6000 rpm for 15 min

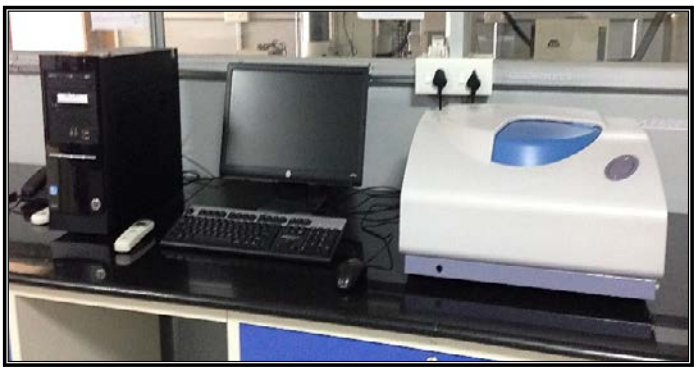
Step 5: Collecting the pellets for keeping in oven at 60^{o}C overnight

Step 6: Macerating the dried pellets to make it as a fine powder of AgNPs

Plate 2. Instruments used for characterization of green synthesized AgNPs

Soniacator

Particle size analyzer



Uv-Vis Spectroscopy

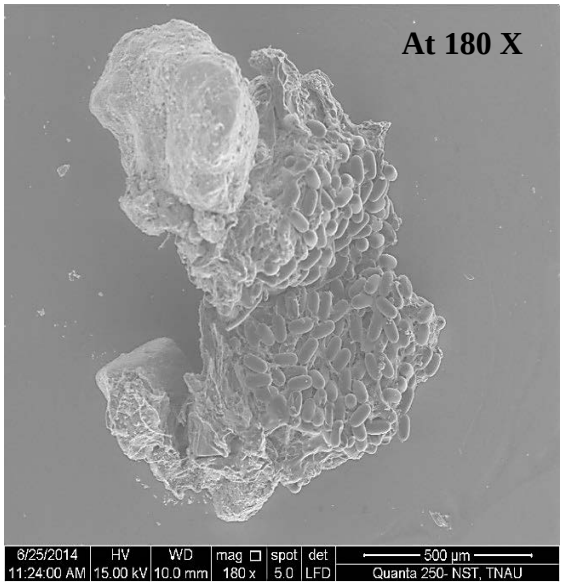
Scanning Electron Microscope (SEM)

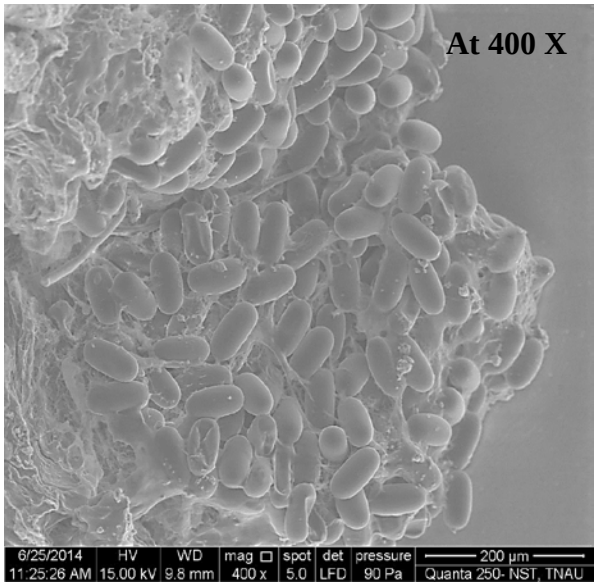
Transmission Electron Microscope (TEM)

X-Ray Diffractometer

FT-IR Spectroscopy

Plate 21. *M. incognita* egg mass at different magnifications of SEM





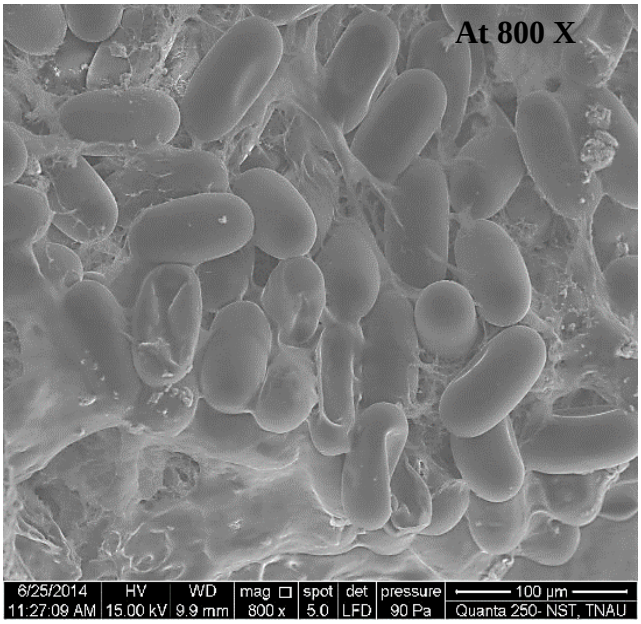
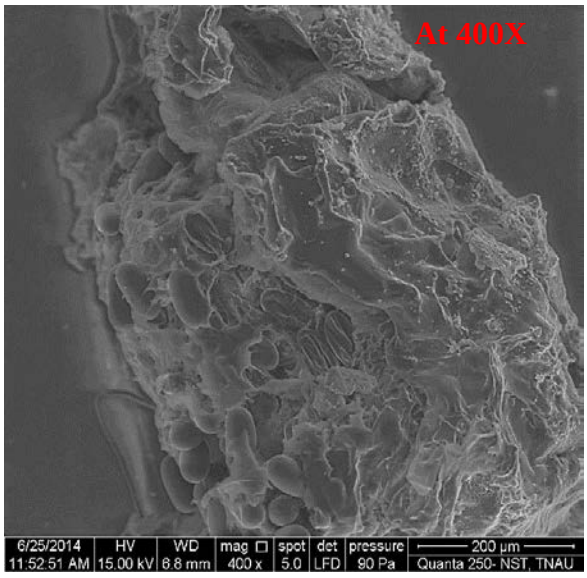
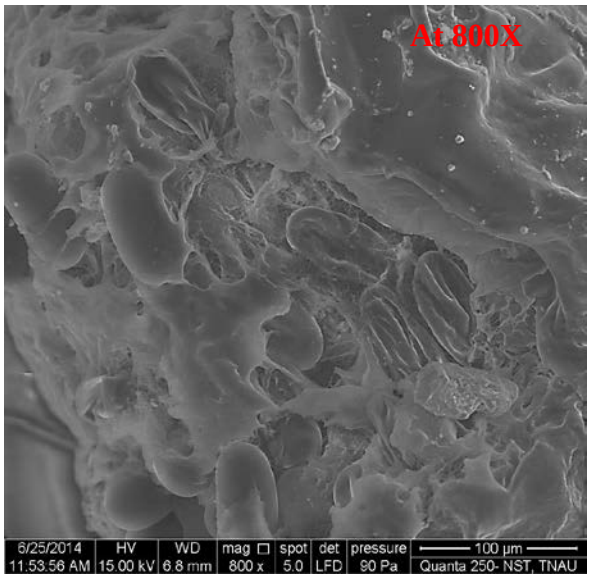


Plate 22. *M. incognita* egg mass treated with AgNPs at different magnifications of SEM





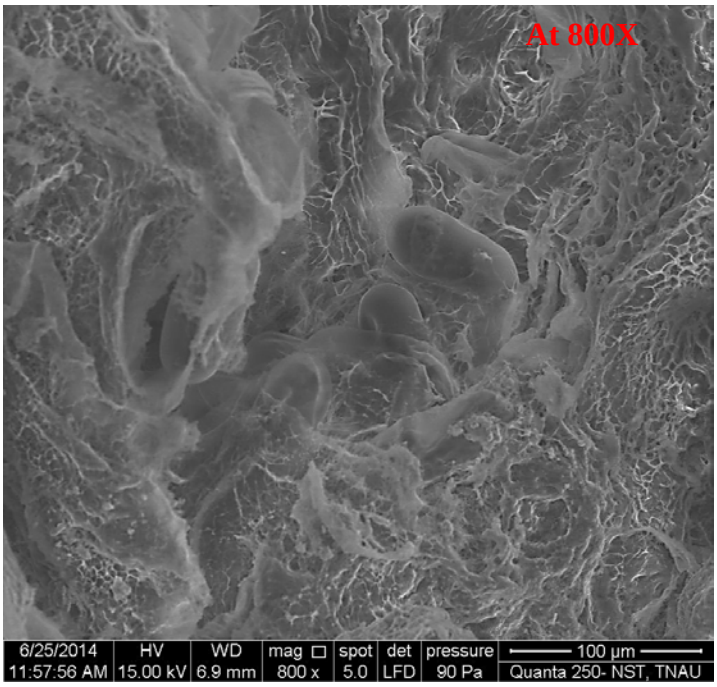
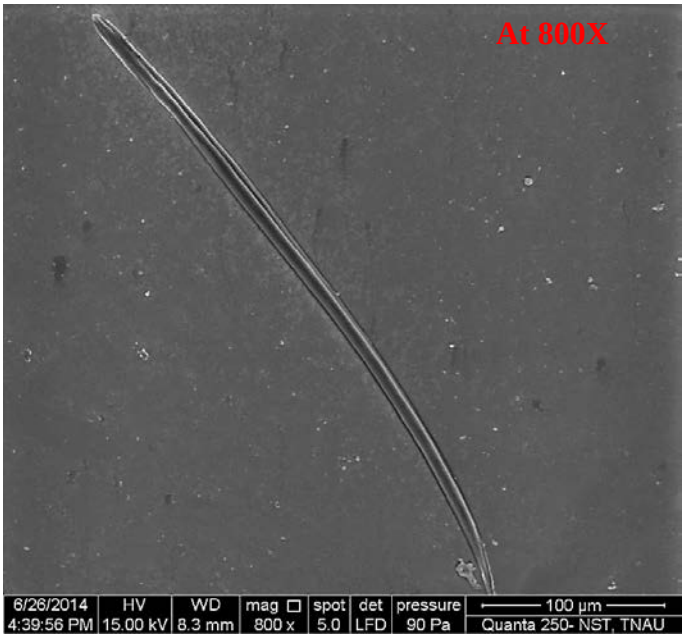


Plate 23. Morphological changes caused by AgNPs on juveniles of *M. incognita*

Untreated



Treated

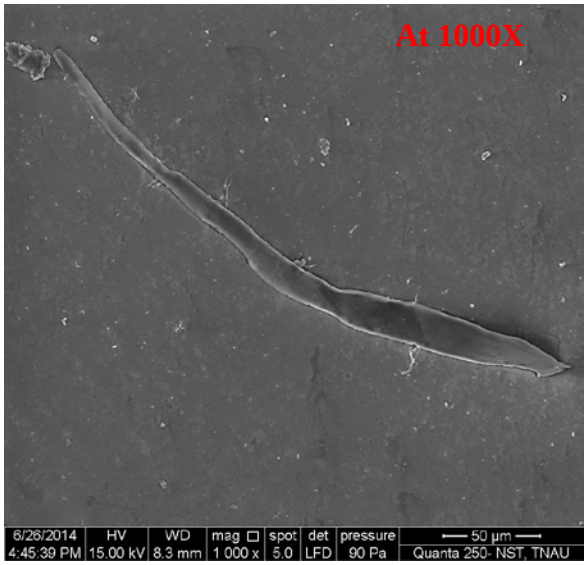
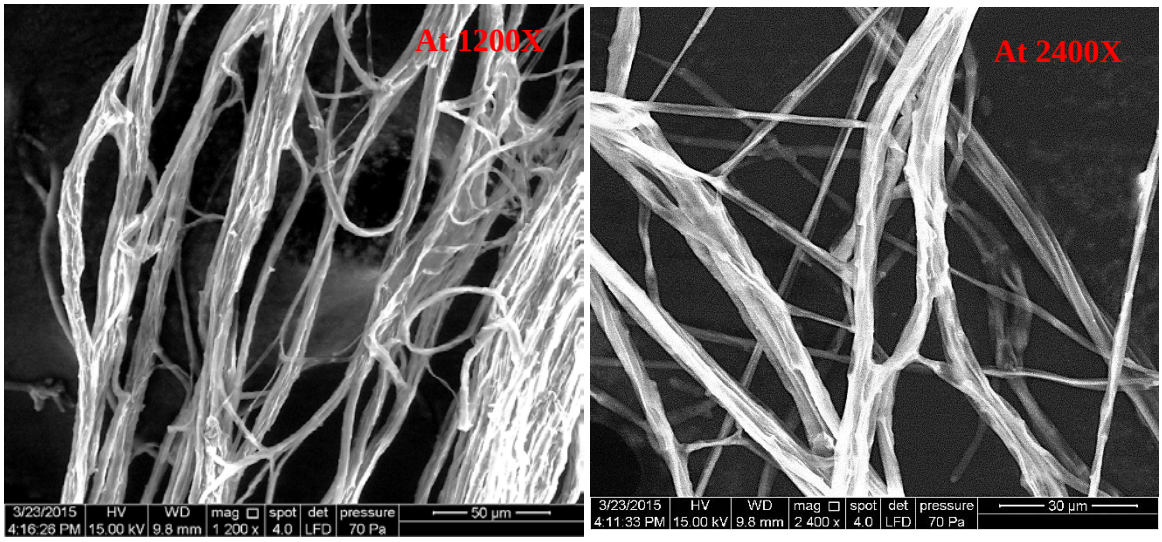
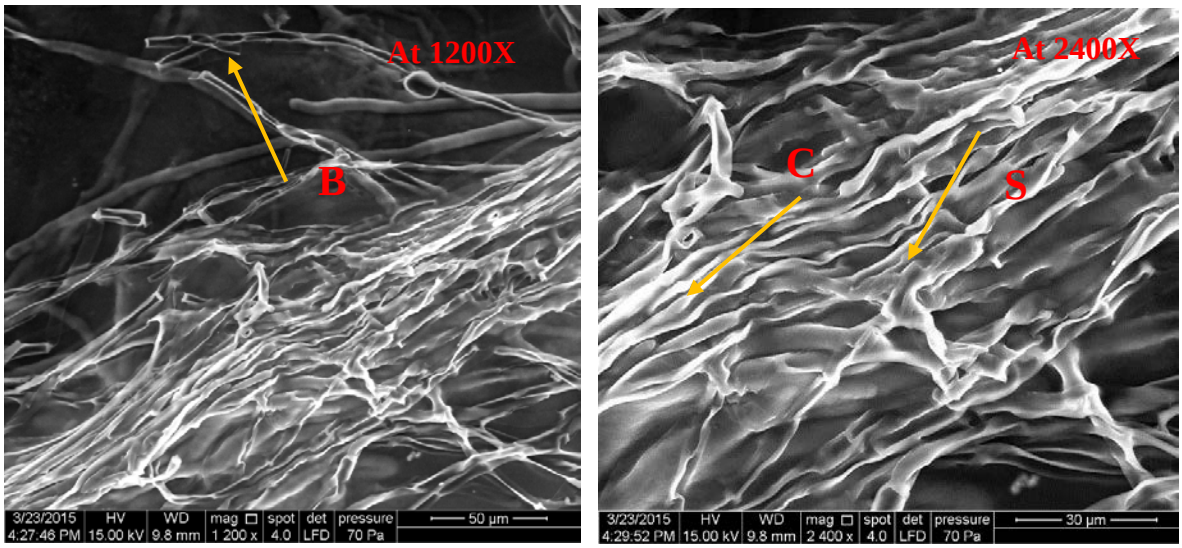


Plate 24. Morphological changes caused due to AgNPs on *F. oxysporum* f.sp. *lycopersici*

Untreated



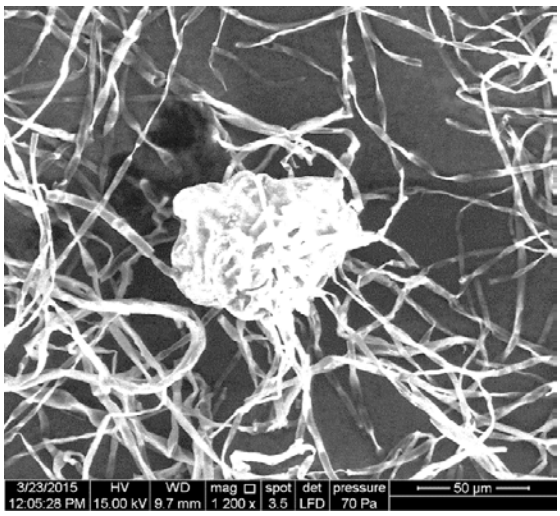
Treated with green synthesized AgNPs

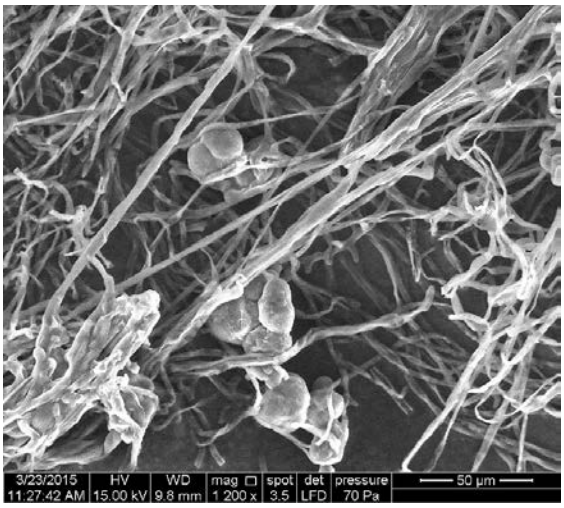


B: Breaking; C: Crinkling and S: Shriveling of mycelia

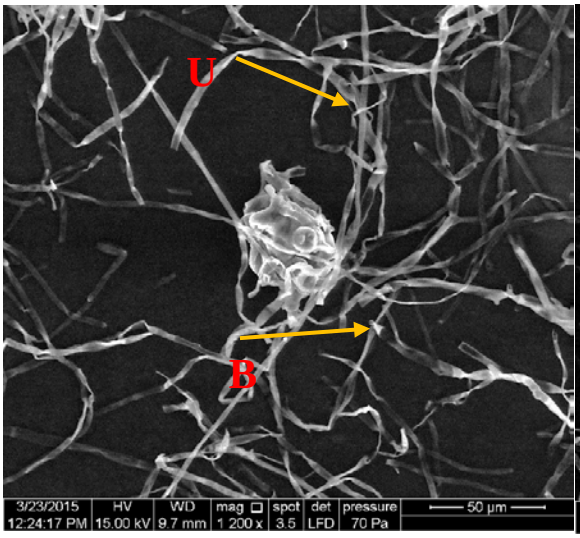
Plate 25. Morphological changes caused by AgNPs on *M. phaseolina*

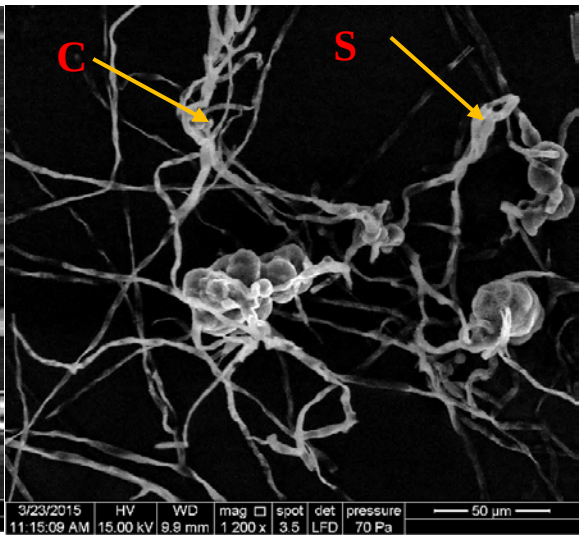
Untreated control





Treated with AgNPs





U: Uneven growth; B: Breaking; C: Crinkling and S: Shriveling of mycelia at 1200X magnification

Plate 17. Inhibitory effect of green synthesized AgNPs *in vitro* on mycelial growth of *F. oxysporum* f.sp. *lycopersici*

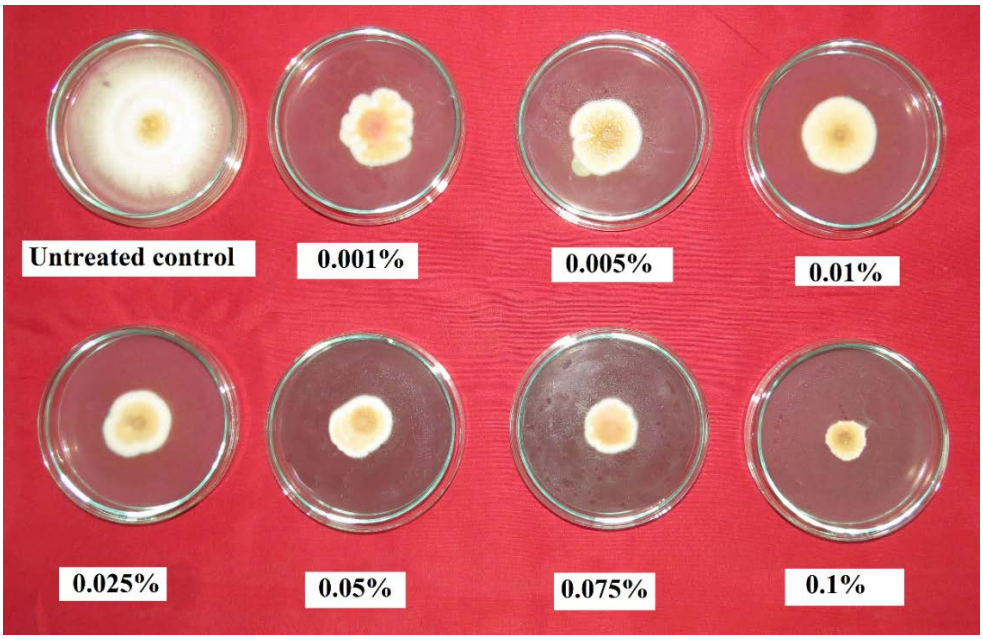


Plate 18. Inhibitory effect of green synthesized AgNPs *in vitro* on mycelial growth of *M. phaseolina*

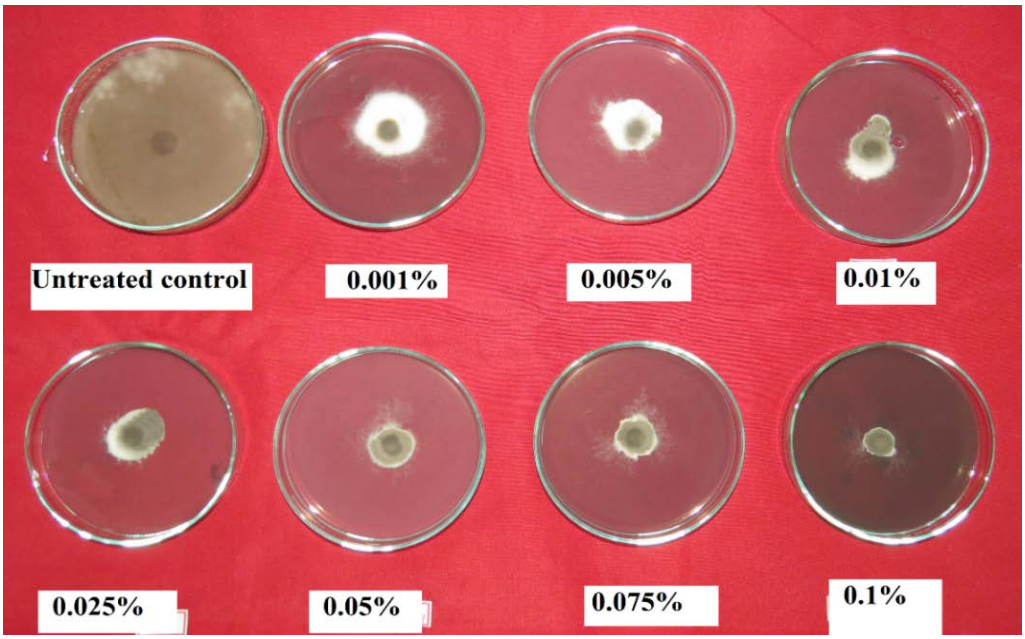


Plate 19. Inhibitory effect exhibited by green synthesized AgNPs on *R. solanacearum* in agar well diffusion method

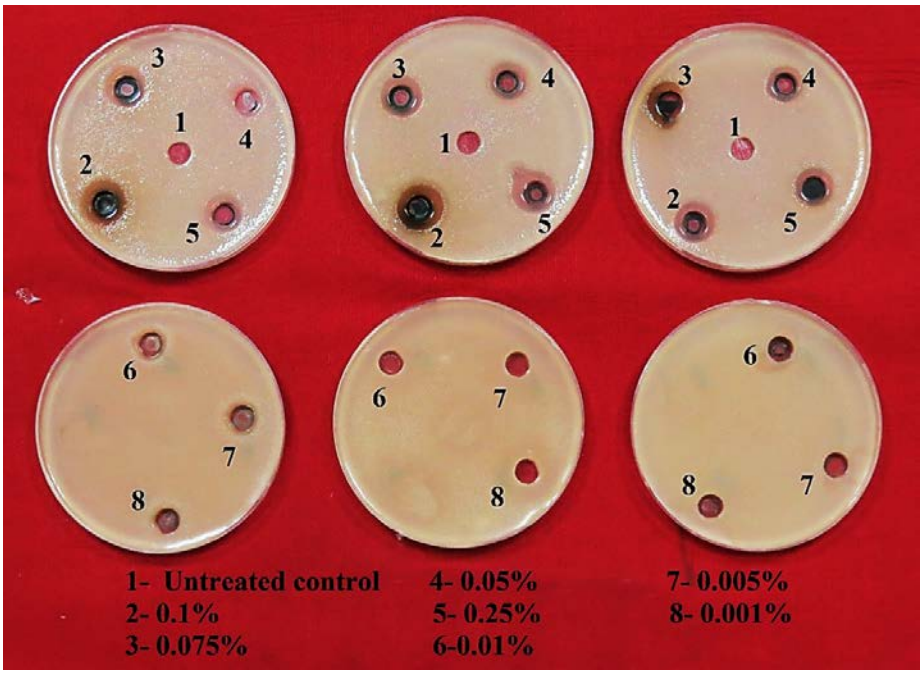


Plate 20. Growth inhibition caused by green synthesized AgNPs on *X. oryzae* pv. *oryzae* in agar well diffusion method

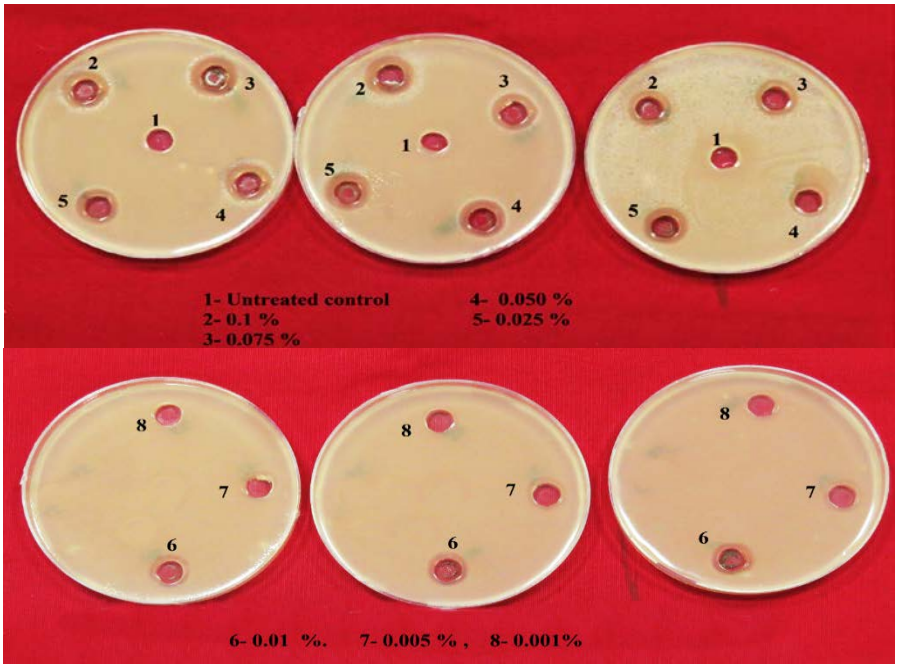


Plate 3. Management of root knot nematode on tomato under glasshouse conditions: Experimental view

Plate 4. View of experiment conducted for management of Fusarium wilt on tomato under glasshouse conditions

Plate 5. View of experiment conducted for management of charcoal rot on blackgram under glasshouse conditions

Plate 6. Management of bacterial wilt of binjal under glasshouse conditions: Experimental view

Plate 7. View of experiment conducted for management of nematode fungal disease complex in tomato under glasshouse conditions

Plate 8. View of experiment on management of nematode fungal disease complex in blackgram under glasshouse conditions

Plate 9. Management of nematode bacterial disease complex in brinjal under glasshouse conditions: Experimental view

Plate 26. Effect of green synthesized AgNPs on plant growth of tomato challenged with *M. incognita*

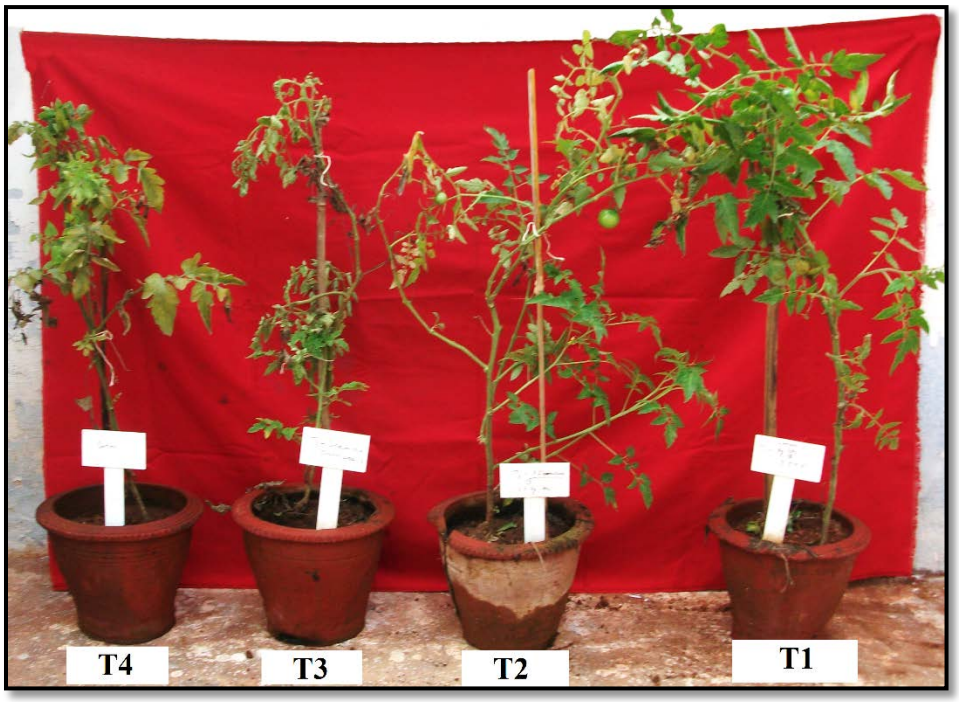


Plate 27. Influence of green synthesized AgNPs on root biomass of tomato inoculated with *M. incognita*

Plate 28. Effect of green synthesized AgNPs on plant growth of tomato challenged with *F. oxysporum.* f. sp. *lycopersici*

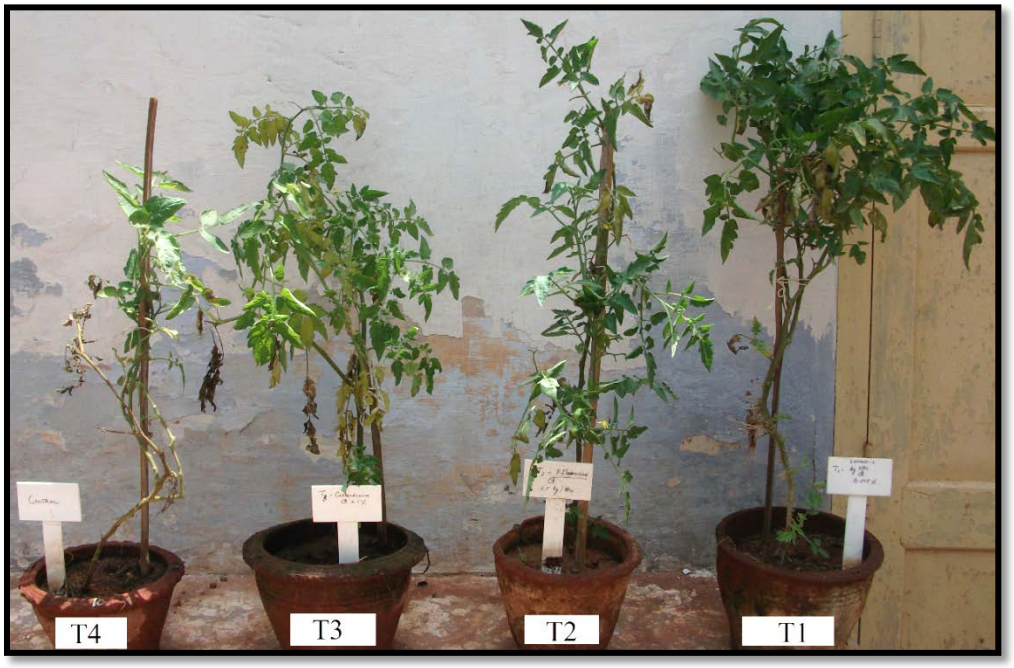


Plate 29. Variations among treatments on plant stand of blackgram challenged with *M. phaseolina*

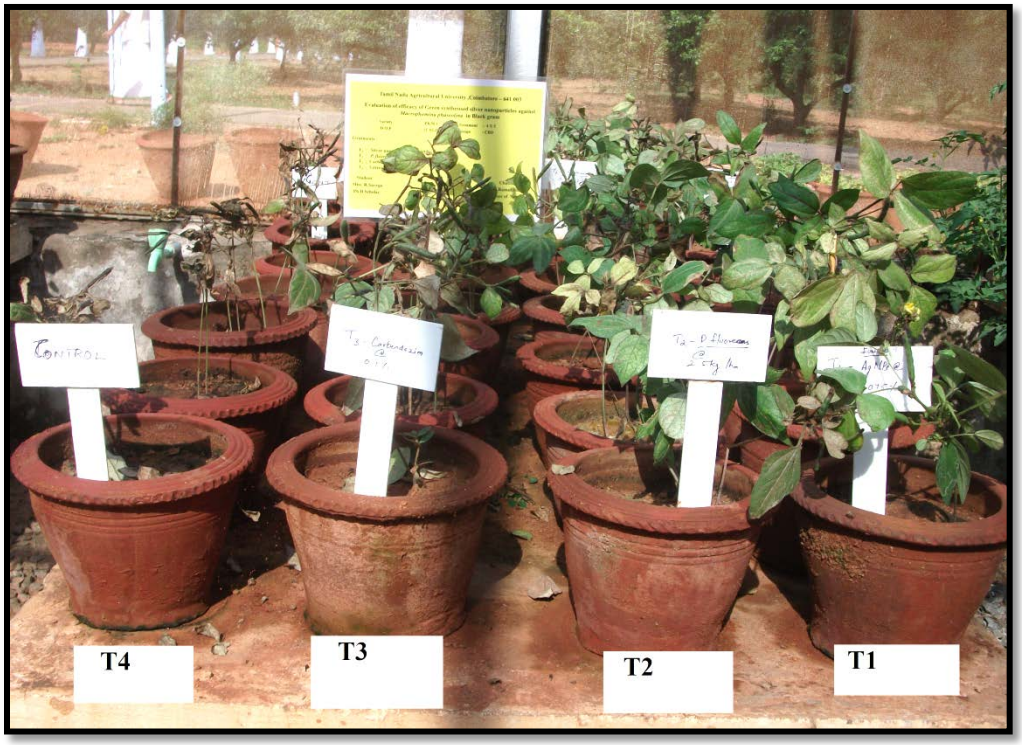


Plate 30. Enhancement in plant growth characters of brinjal inoculated with *R. solanacearum* following the treatment of green synthesized AgNPs

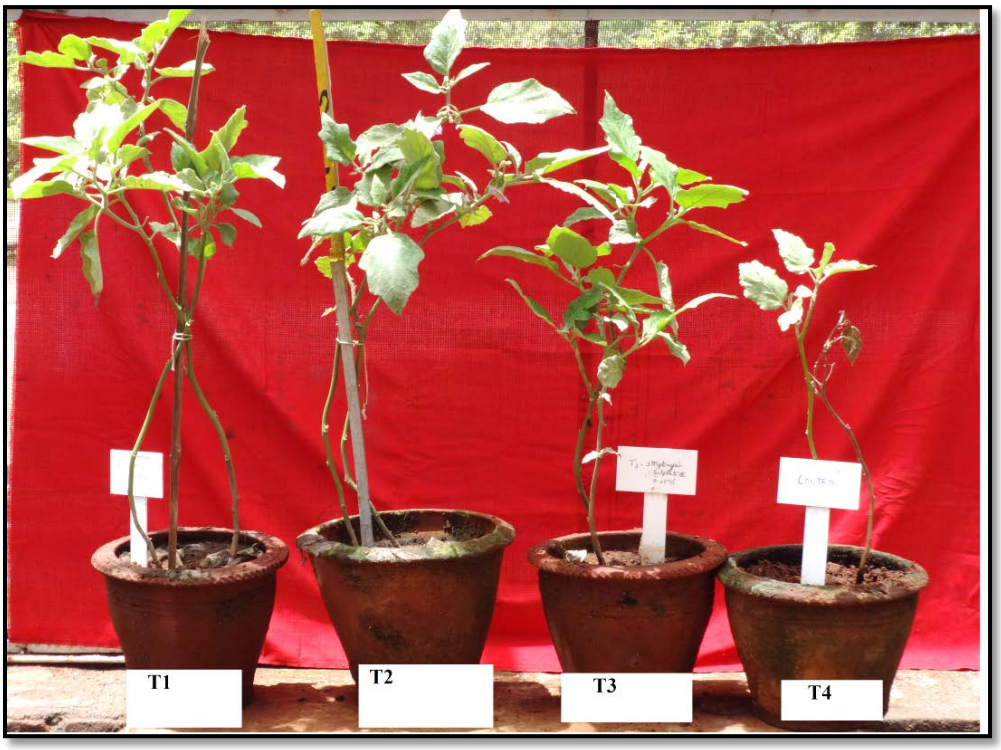


Plate 31. Variations in plant growth among treatments in tomato affected by nematode fungal disease complex

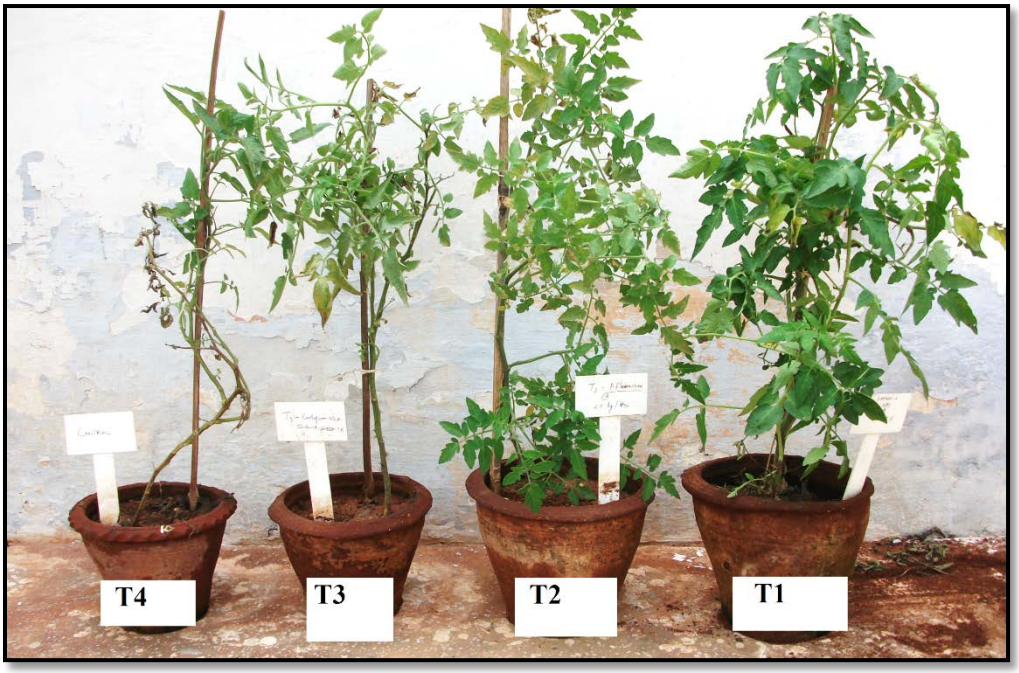


Plate 32. Improvement in plant growth due to suppression of nematode fungal disease complex of blackgram by green synthesized AgNPs

Plate 33. Improvement in plant growth due to suppression of nematode bacterial disease complex of brinjal by green synthesized AgNPs

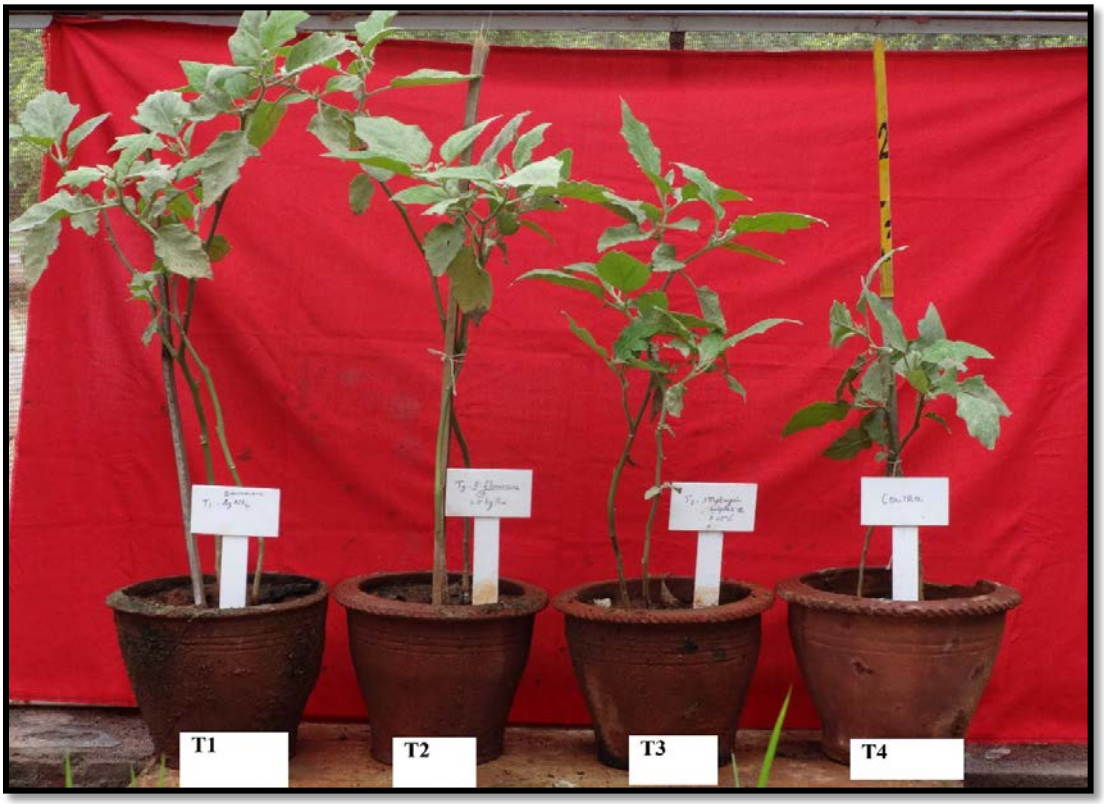


Plate 10. View of experiments conducted to reconfirm the effect of green synthesized AgNPs on different crops in plant growth chamber

Plate 34. Effect of green synthesized AgNPs on juveniles of *Steinernema glaseri*

Untreated **Treated**

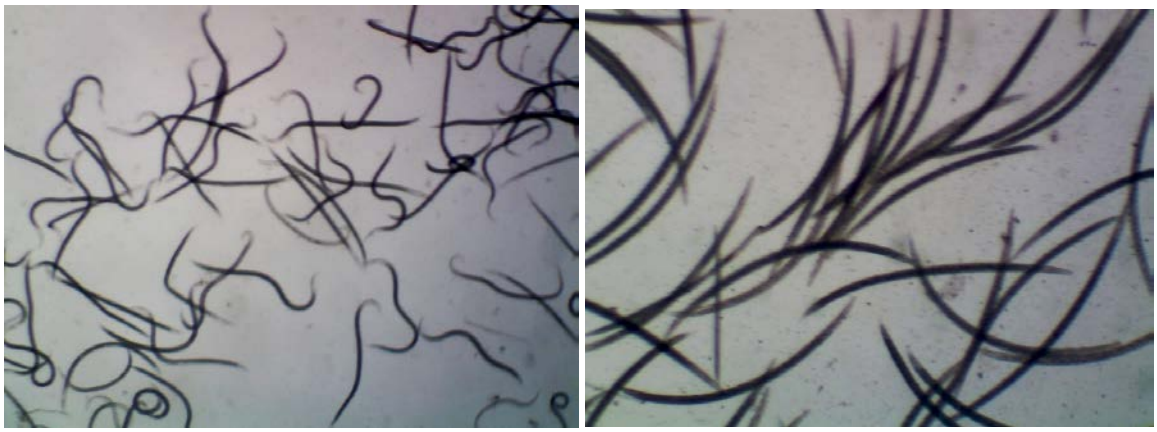


Plate 35. Inhibitory effect of green synthesized AgNPs on *T.viride*

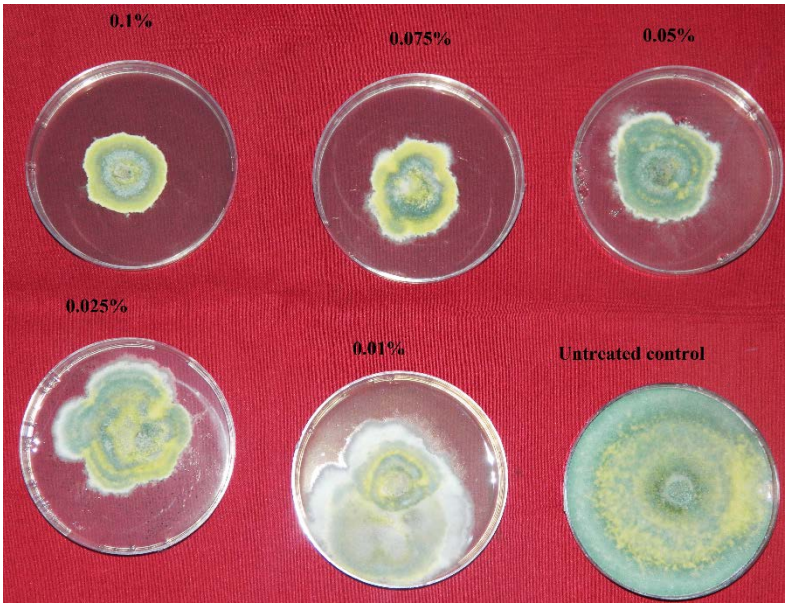


Plate 36. Inhibitory effect of green synthesized AgNPs on *P.lilacinus*

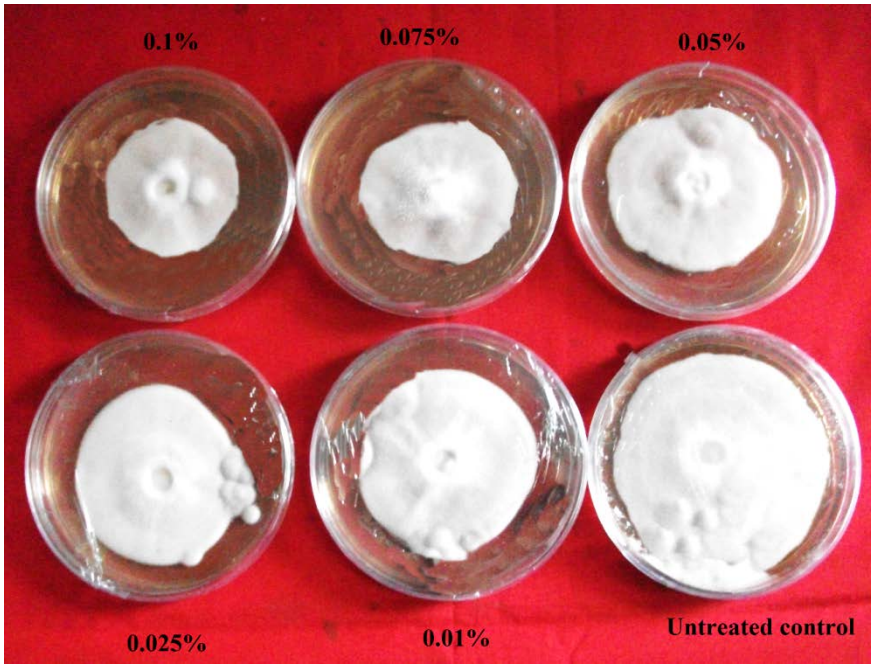


Plate 37. Inhibitory effect of green synthesized AgNPs on *P. fluorescens*

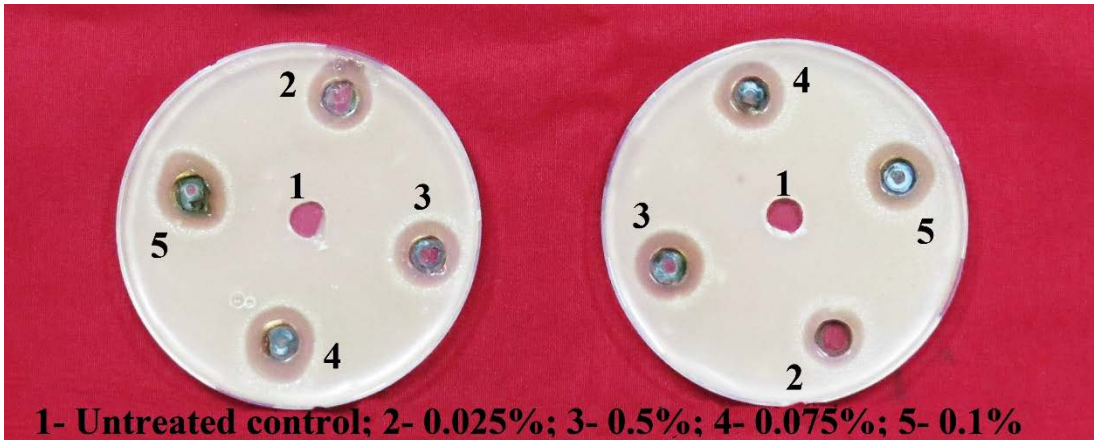


Plate 38. Inhibitory effect of green synthesized AgNPs on *B. subtilis*

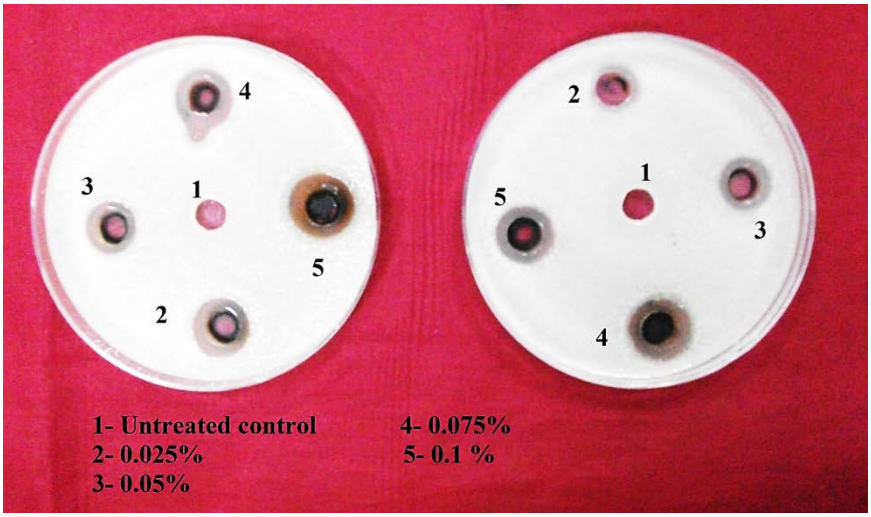


Figure 8. UV-Vis spectra analysis for synthesised AgNPs using *T.procumbens*

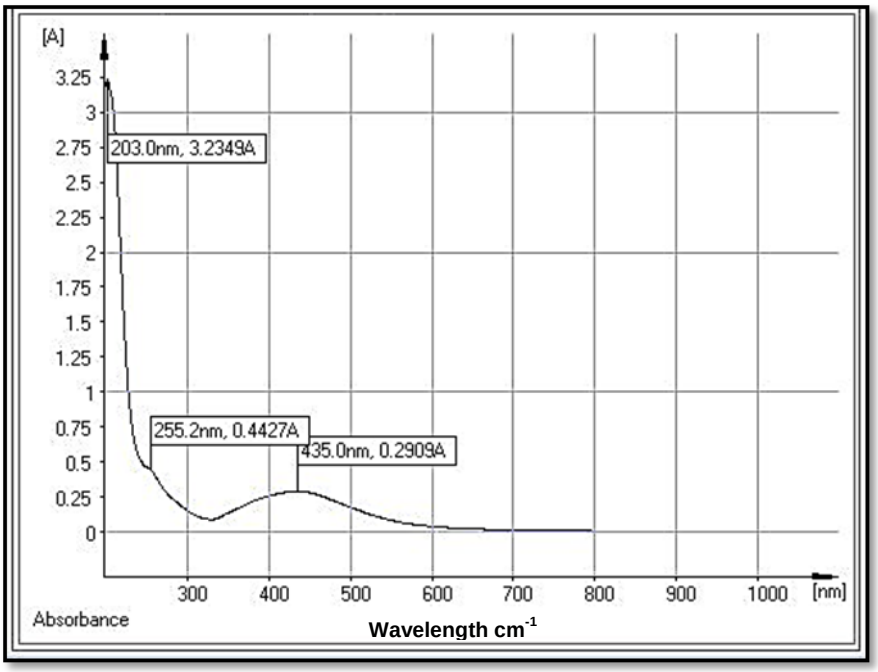


Figure 9. UV-Vis spectra analysis for synthesised AgNPs using *E.hirta*

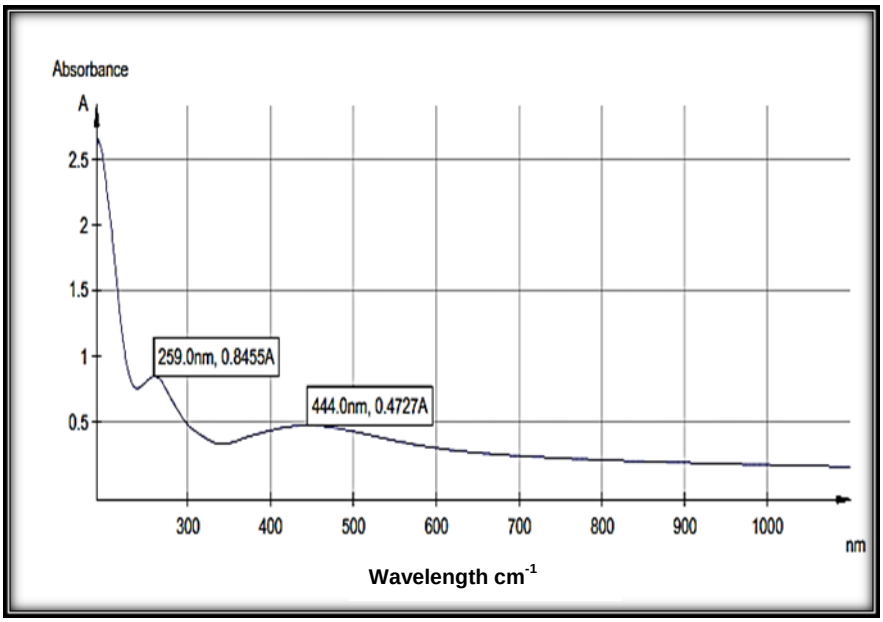


Figure 10. UV-Vis spectra analysis of AgNPs green synthesised using *A.indica*

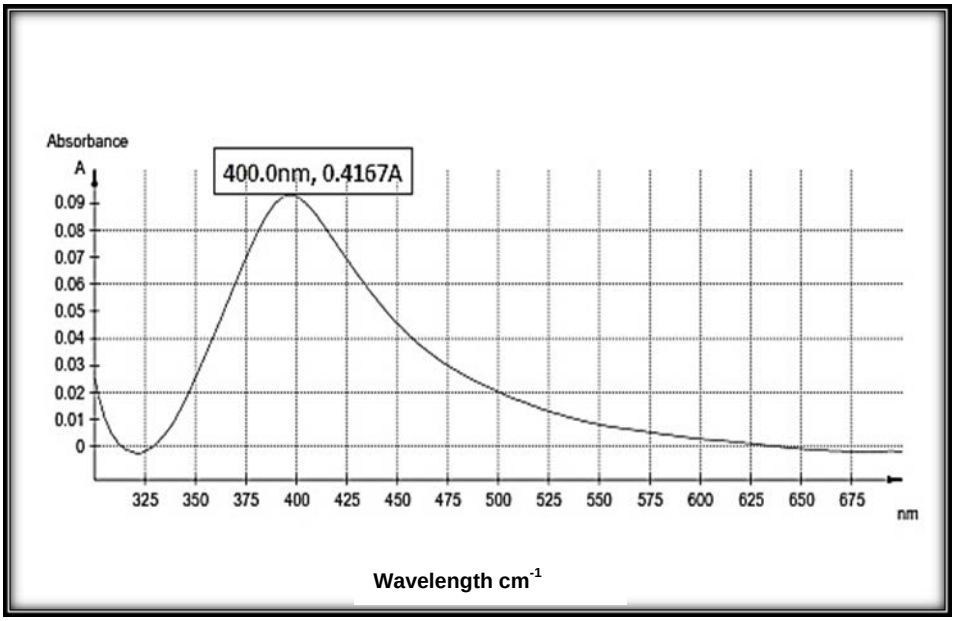
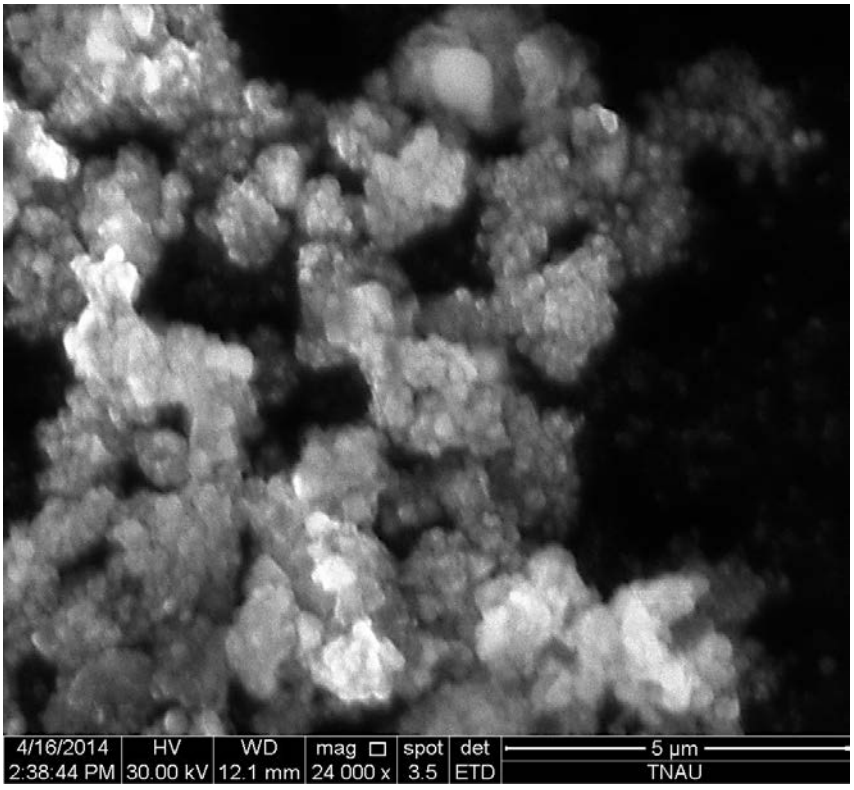


Plate 11. Image of *T. procumbens* mediated green synthesized AgNPs using SEM with EDAX



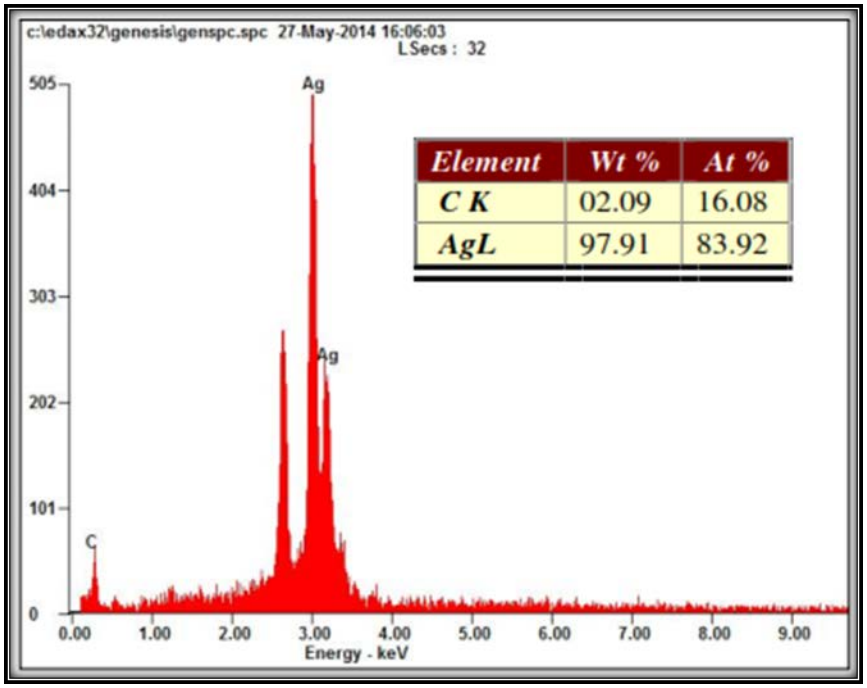
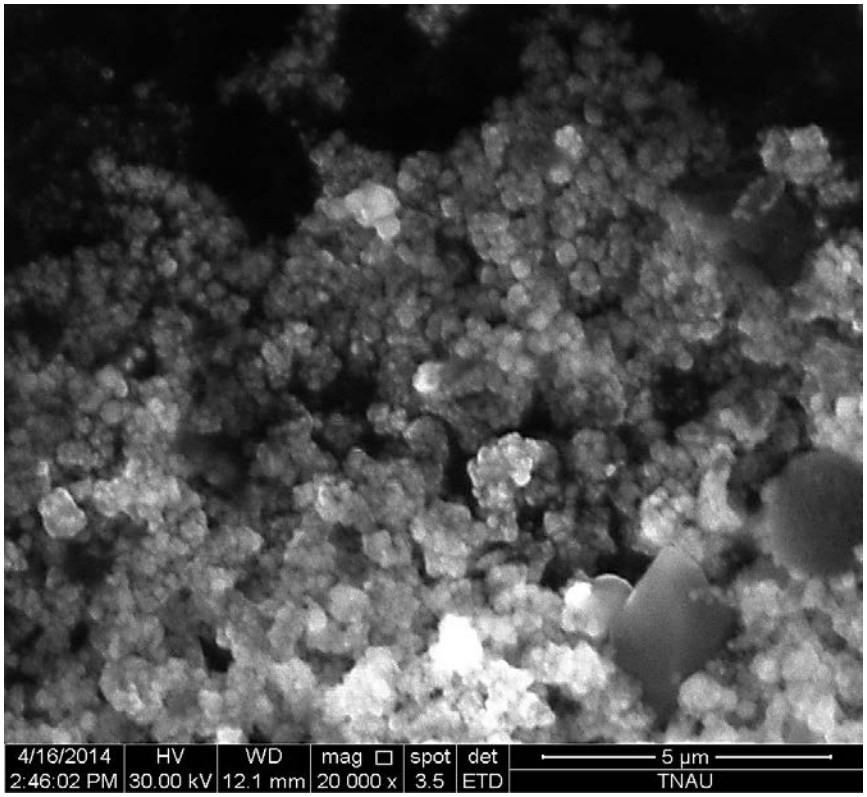


Plate 12. Image of *E. hirta* mediated green synthesized AgNPs using SEM with EDAX



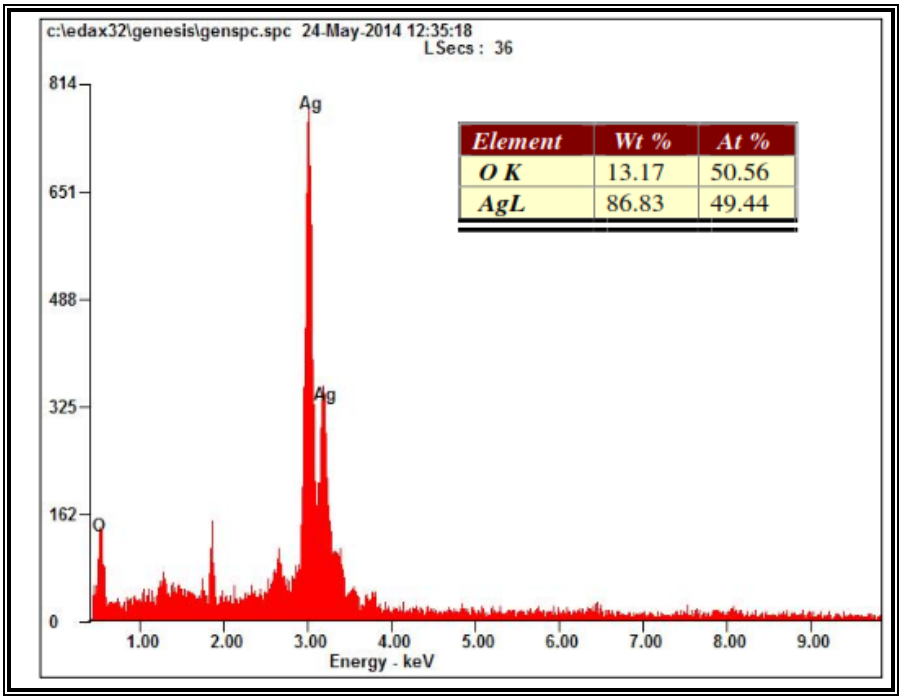
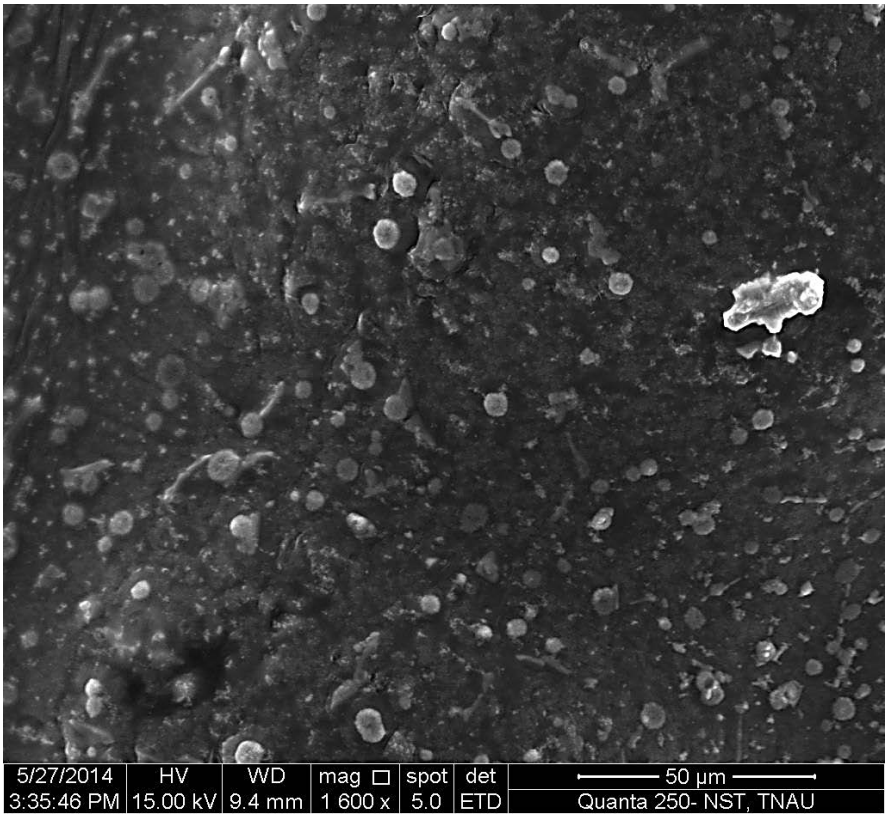


Plate 13. SEM with EDAX image of *A. indica* mediated green synthesized AgNPs



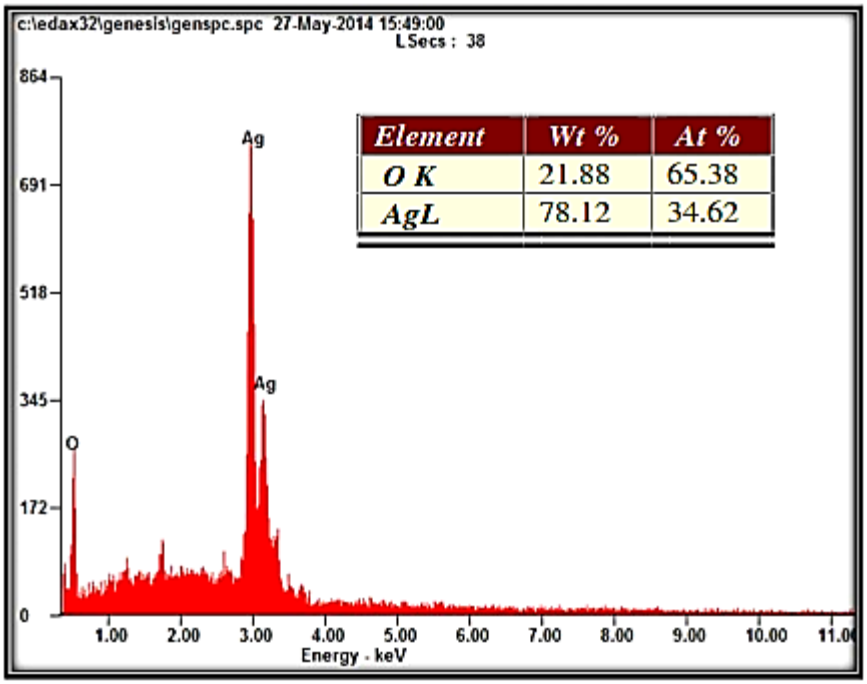
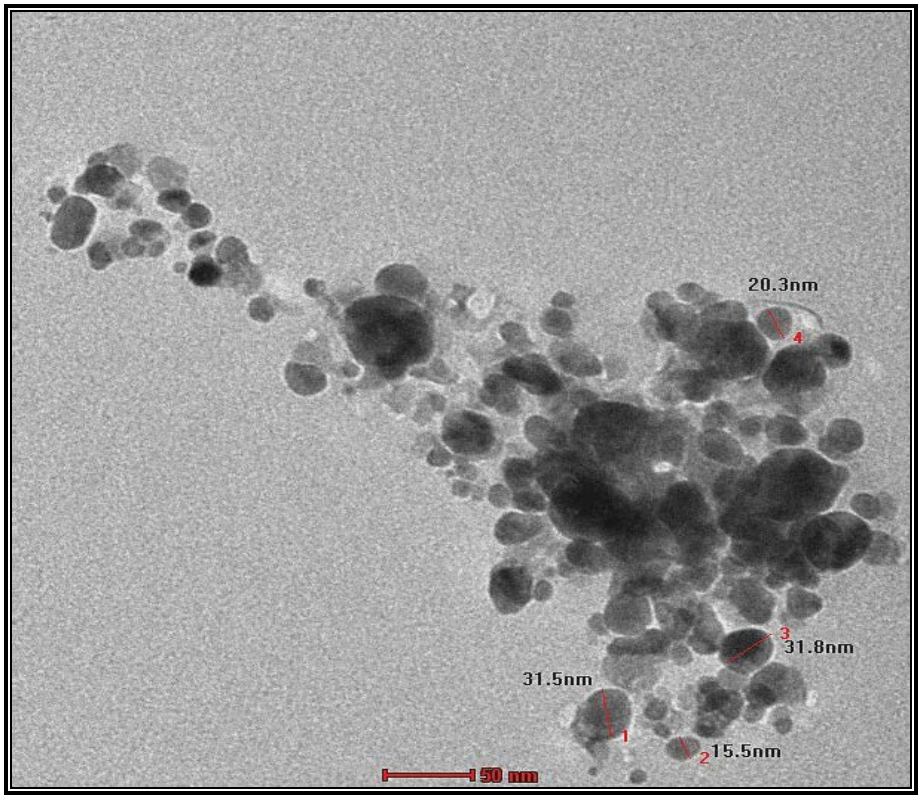


Plate 14. TEM with EDAX image of green synthesized AgNPs with *T. procumbens*



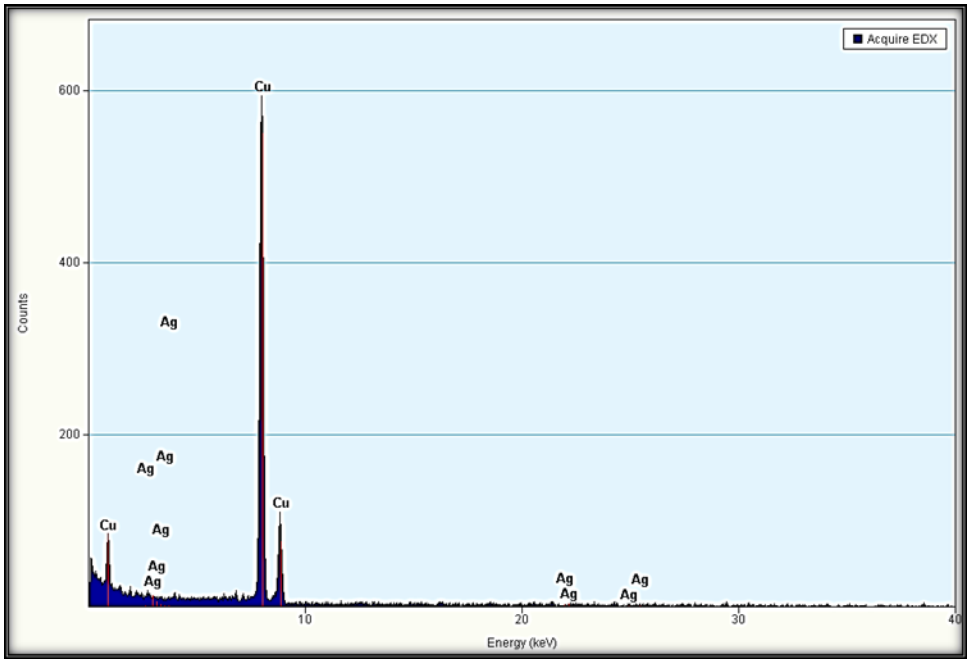
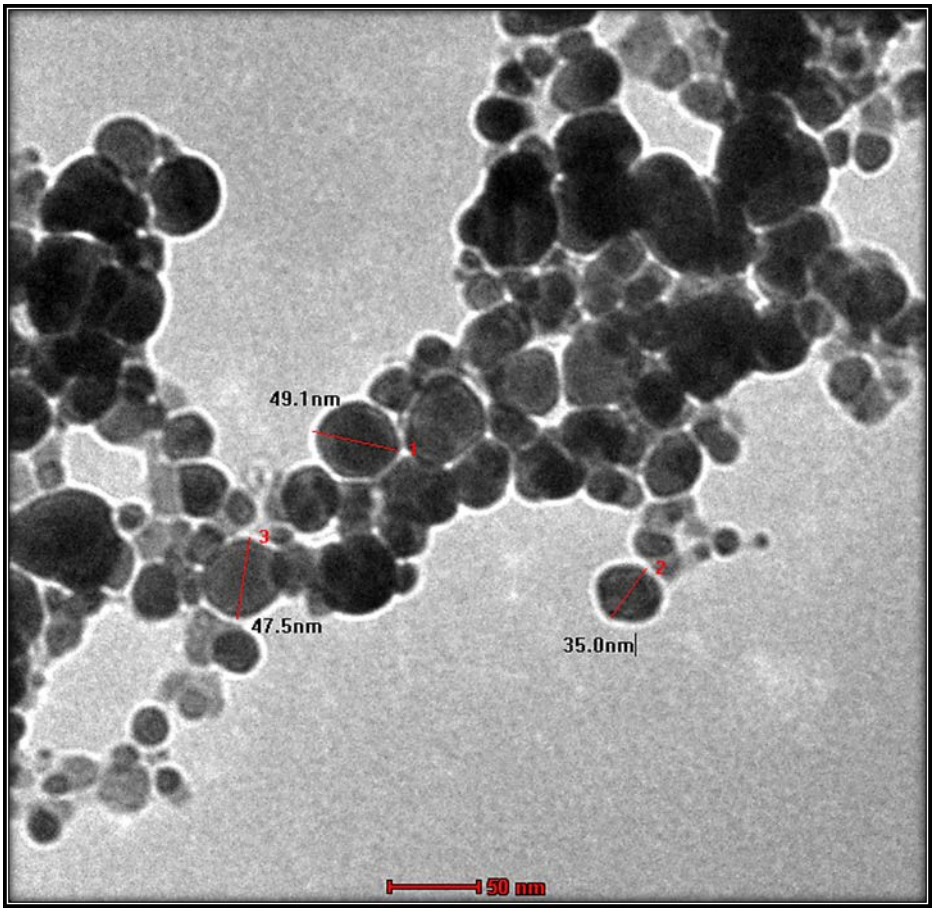


Plate 15. TEM with EDAX image of green synthesized AgNPs using *E. hirta*



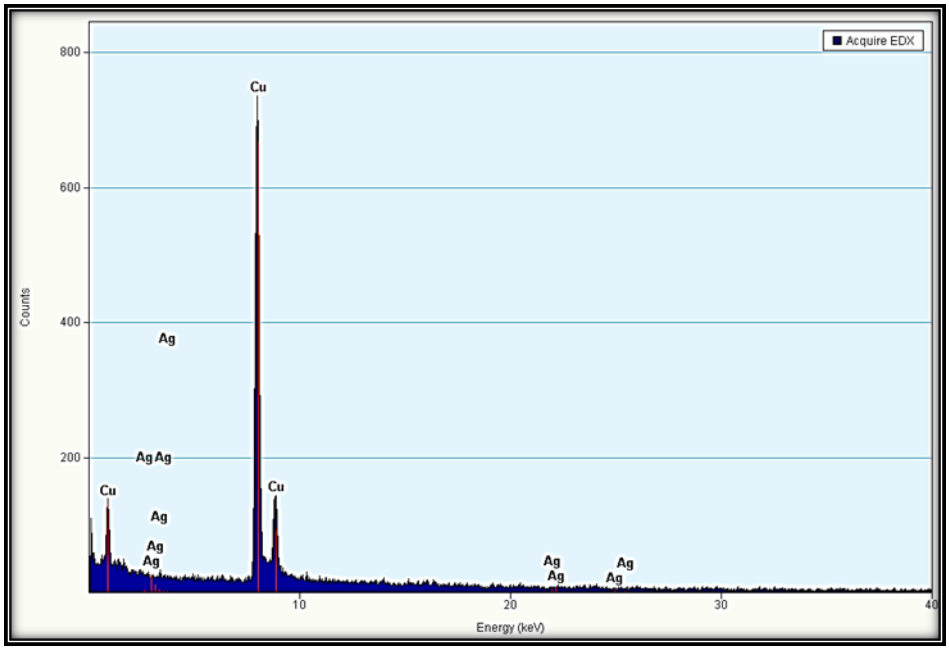
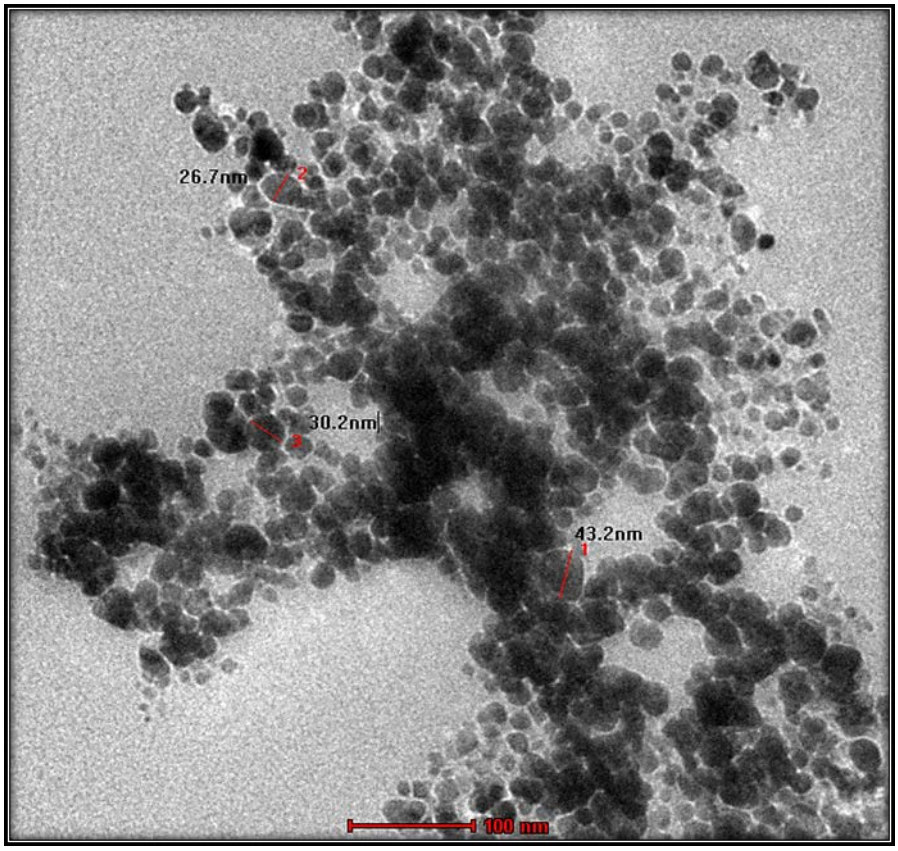


Plate 16. TEM with EDAX image of green synthesized AgNPs using *A. indica*



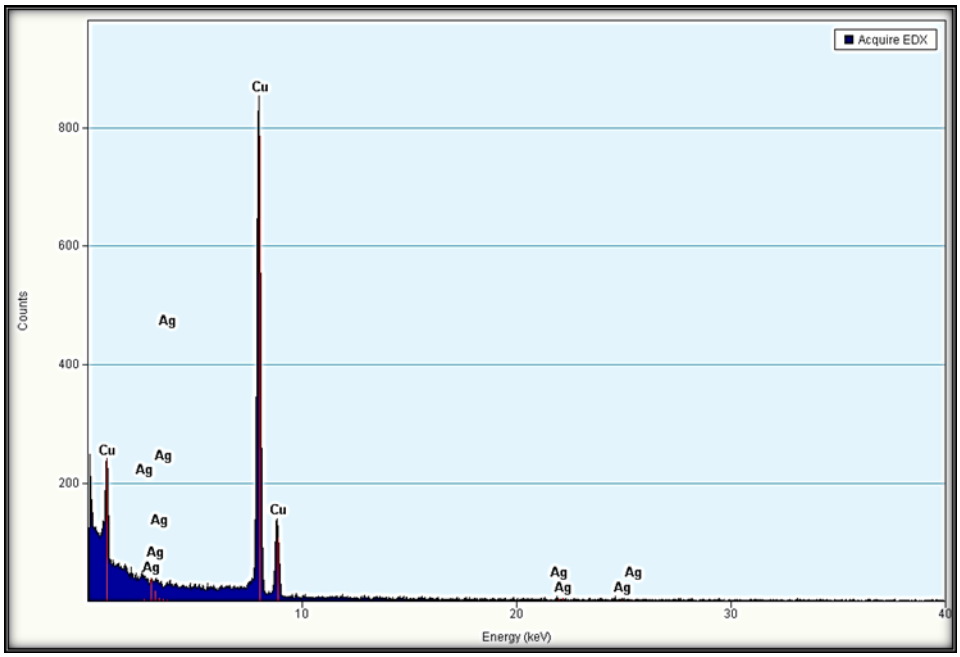


Figure 11. XRD pattern of green synthesized AgNPs using *T. procumbens*

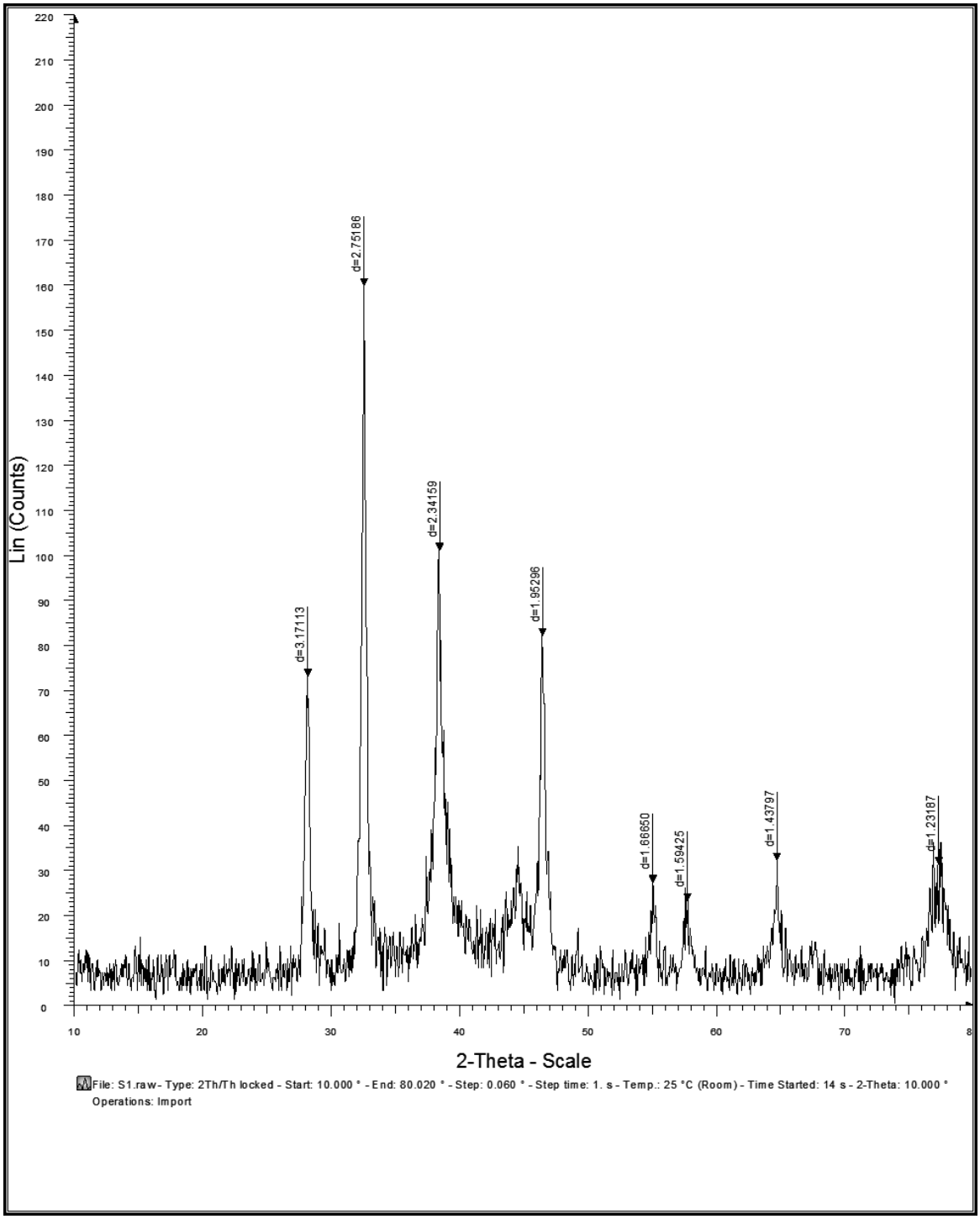


Figure 12. XRD pattern of green synthesized AgNPs with *E. hirta*

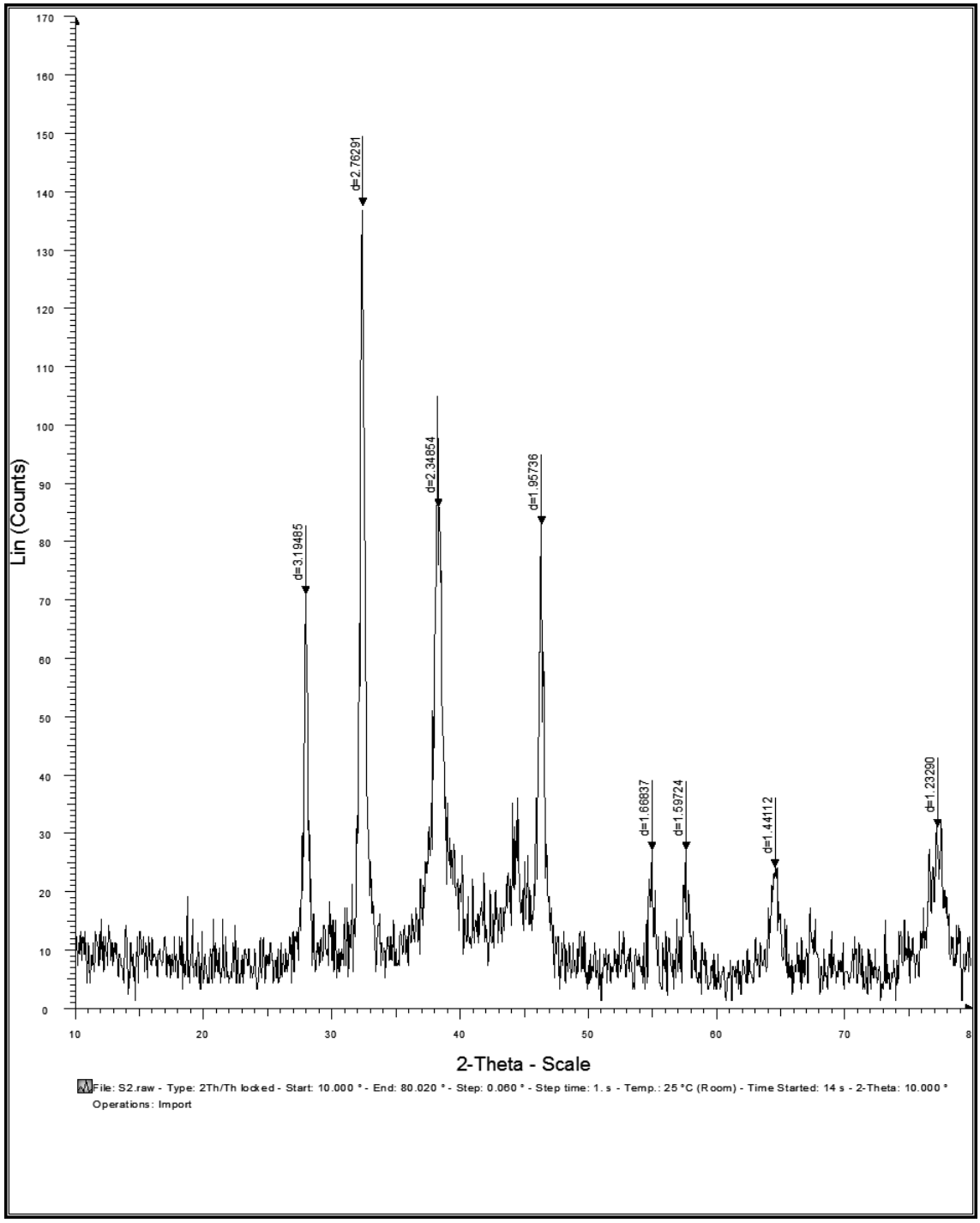


Figure 13. XRD pattern of green synthesized AgNPs with *A. indica*

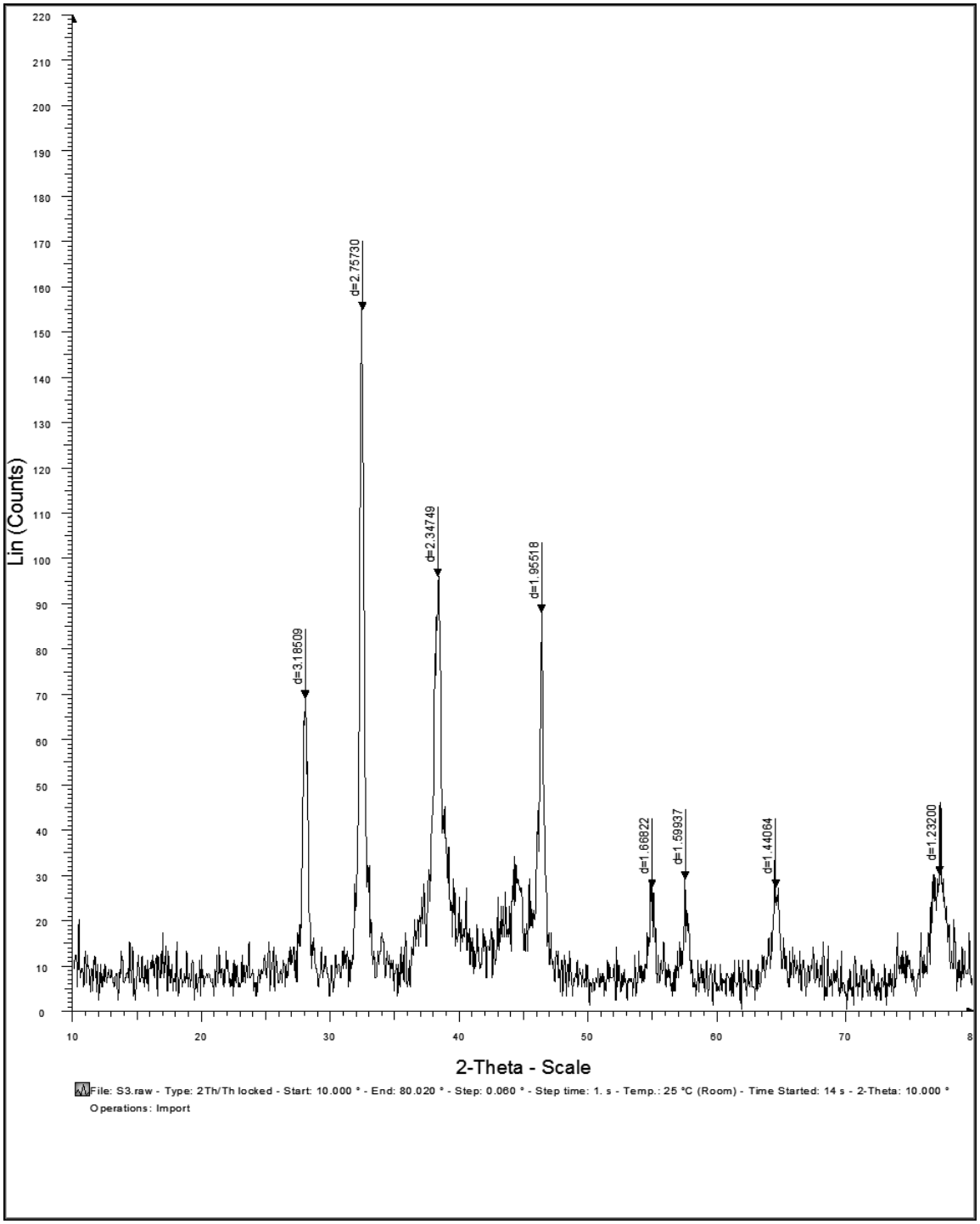


Figure 14. Spectral analysis of *T. procumbens* mediated green synthesized AgNPs using FTIR

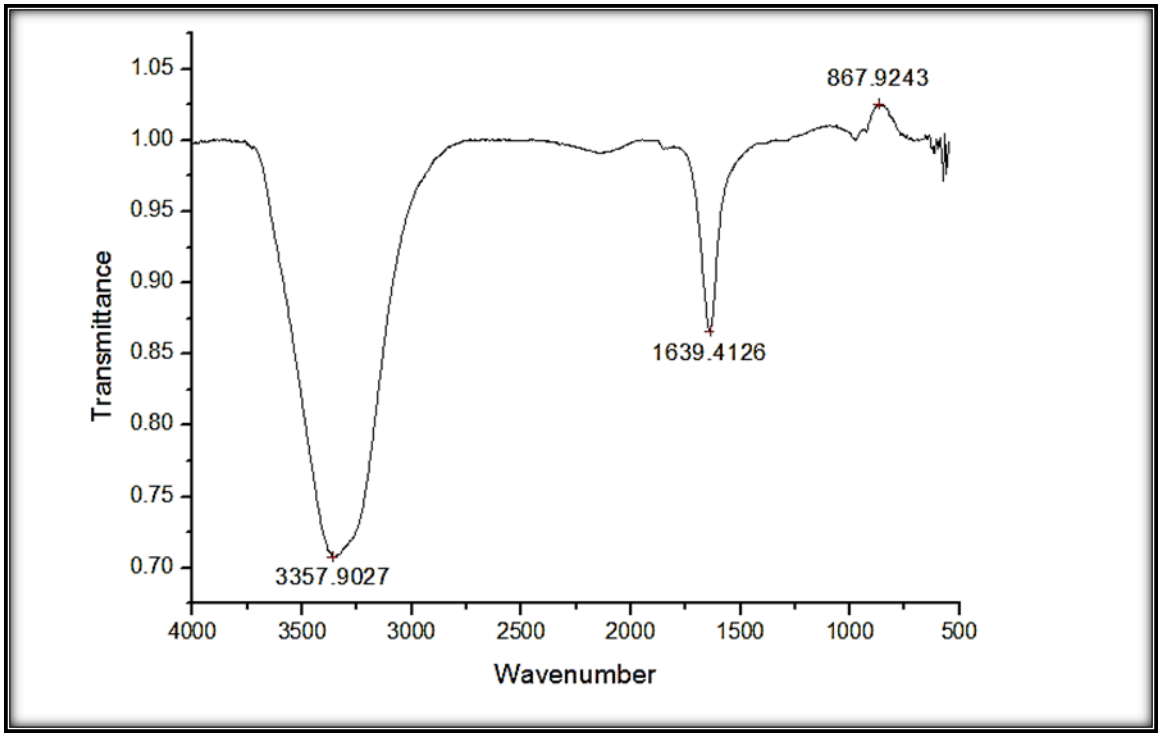


Figure 15. Spectral analysis of *E. hirta* mediated green synthesized AgNPs using FTIR

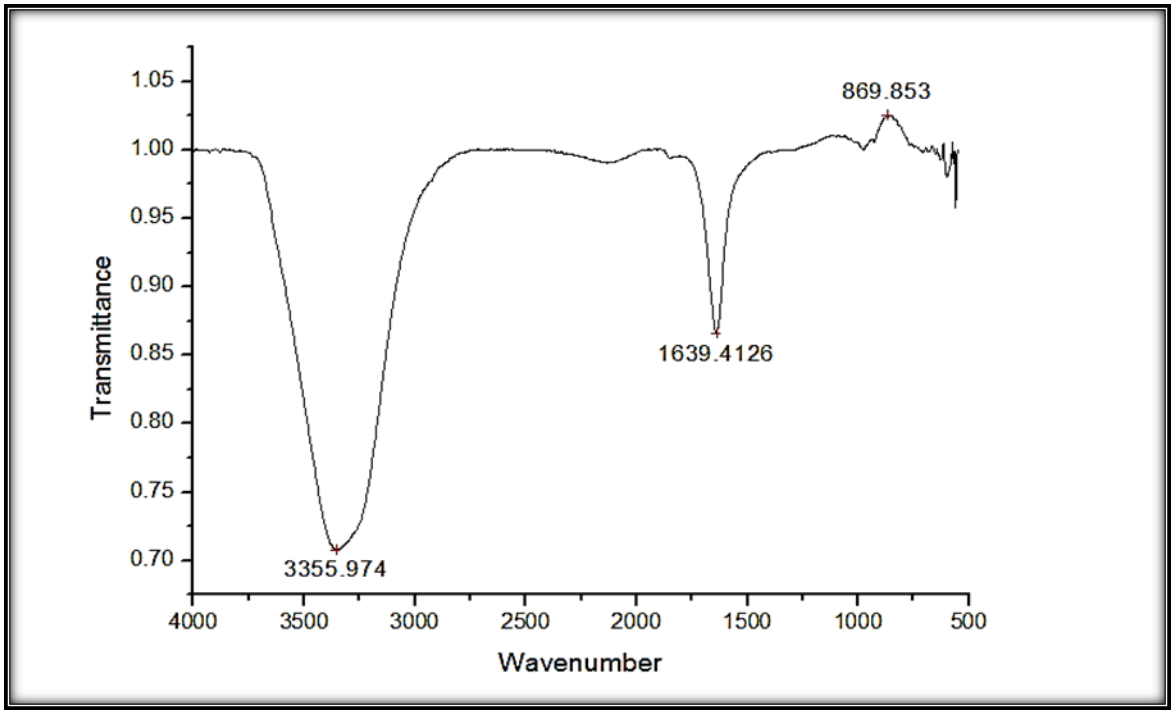


Figure 16. Spectral analysis of *A. indica* mediated green synthesized AgNPs using FTIR

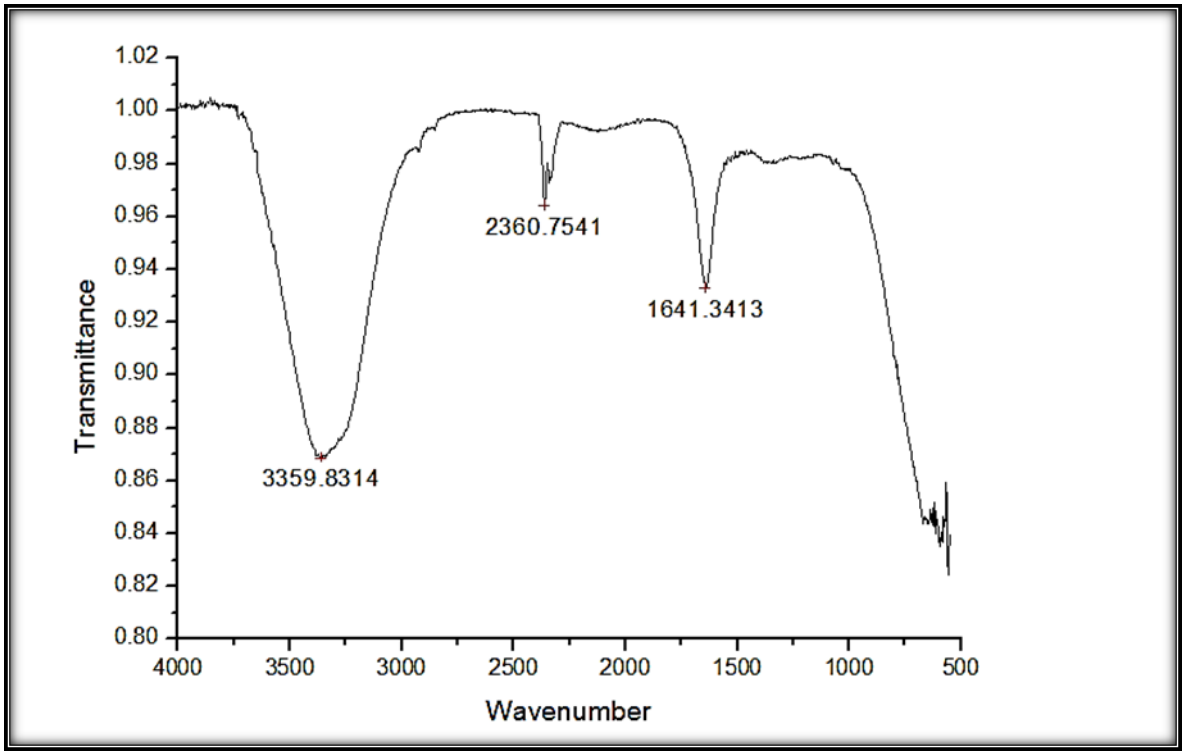


Figure 2. Particle size distribution of green synthesized AgNPs using *T. procumbens*

Tridax.nsz

Measurement Results

Date	:	Tuesday, November 26, 2013 4:16:04 PM
Measurement Type	:	Particle Size
Sample Name	:	Tridax
Scattering Angle	:	90
Temperature of the holder	:	25.1 °C
T% before meas.	:	20919
Viscosity of the dispersion medium	:	0.893 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	2905 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	78.9 nm	6.7 nm	78.5 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	78.9 nm	6.7 nm	78.5 nm

Cumulant Operations

Z-Average	:	3236.7 nm
PI	:	5.249

Molecular weight measurement

Molecular weight	:	---
Mark-Houwink-Sakurada parameters	:	---

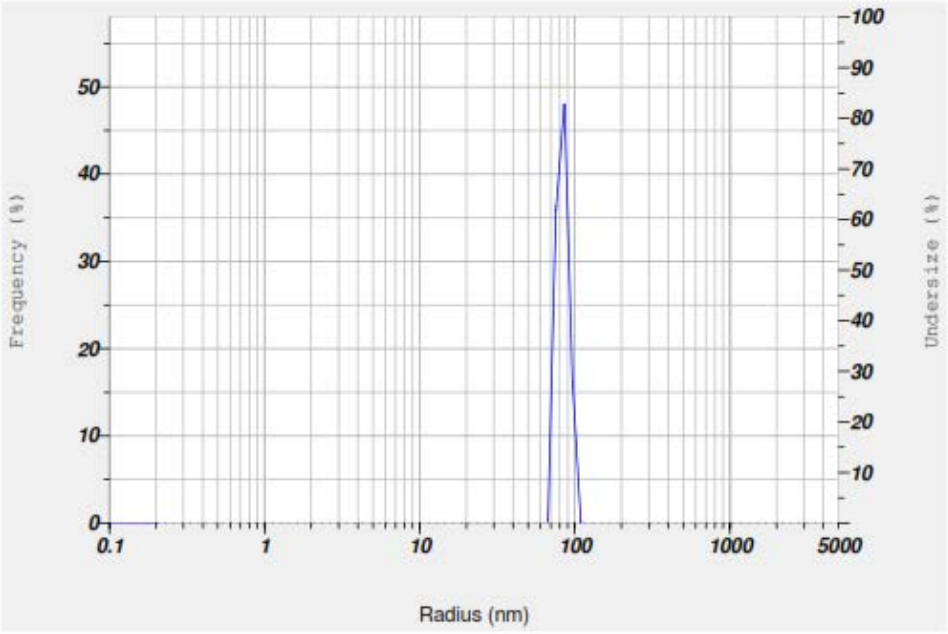


Figure 3. Particle size distribution of *E. hirta* mediated green synthesized of AgNPs

Measurement Results

Date	:	Tuesday, November 26, 2013 4:07:15 PM
Measurement Type	:	Particle Size
Sample Name	:	Euphoria
Scattering Angle	:	90
Temperature of the holder	:	25.1 ℃
T% before meas.	:	14134
Viscosity of the dispersion medium	:	0.893 mPa·s
Form Of Distribution	:	Standard
Representation of result	:	Scattering Light Intensity
Count rate	:	3067 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	87.6 nm	20.4 nm	81.0 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	87.6 nm	20.4 nm	81.0 nm

Cumulant Operations

Z-Average	:	350.0 nm
PI	:	0.569

Molecular weight measurement

Molecular weight	:	---
Mark-Houwink-Sakurada parameters	:	---

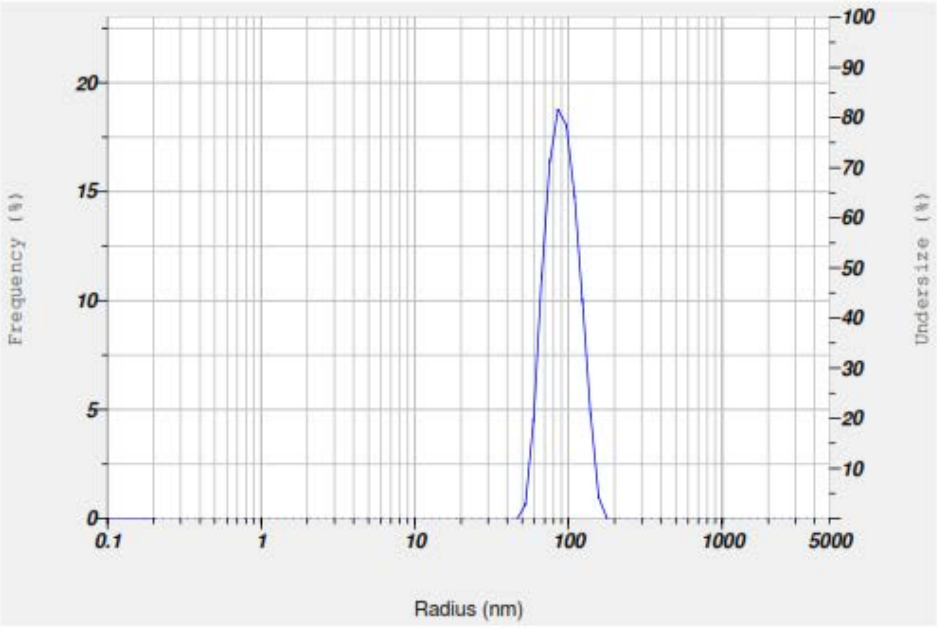


Figure 4. Particle size distribution of green synthesized AgNPs using *A. indica*

Neem.nsz

Measurement Results

Date	: Tuesday, November 26, 2013 4:22:35 PM
Measurement Type	: Particle Size
Sample Name	: Neem
Scattering Angle	: 90
Temperature of the holder	: 25.0 °C
T% before meas.	: 29747
Viscosity of the dispersion medium	: 0.894 mPa·s
Form Of Distribution	: Standard
Representation of result	: Scattering Light Intensity
Count rate	: 1779 kCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	40.4 nm	3.3 nm	39.7 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	40.4 nm	3.3 nm	39.7 nm

Cumulant Operations

Z-Average	: 1921.6 nm
PI	: 2.785

Molecular weight measurement

Molecular weight	: ---
Mark-Houwink-Sakurada parameters	: ---

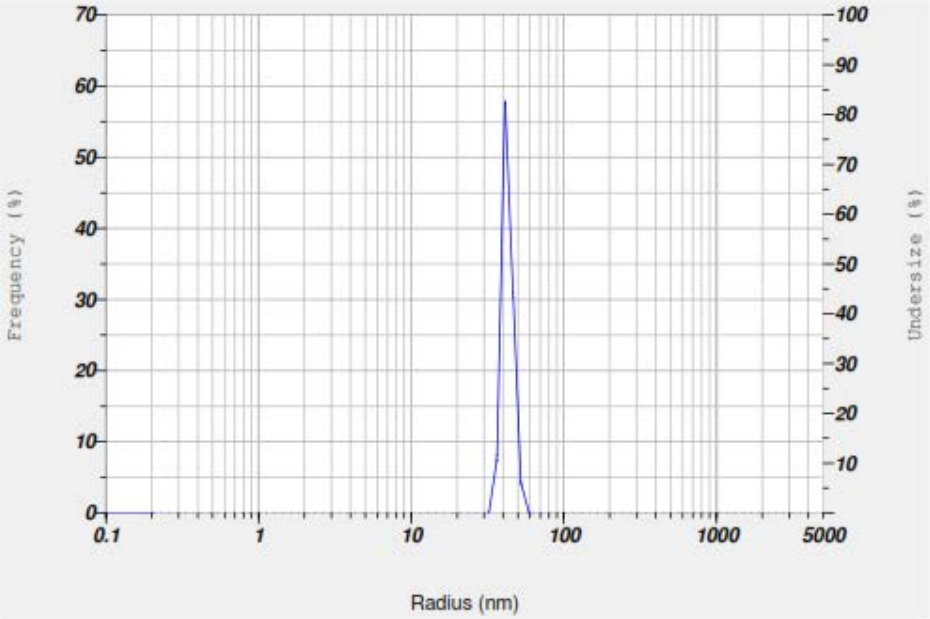


Figure 5. Zeta Potential analysis of green synthesized AgNPs using *T. procumbens*

Measurement Results

Tridax.nzt

Measurement Results

Date	:	Thursday, August 22, 2014 4:05:55 PM
Measurement Type	:	Zeta Potential
Sample Name	:	Tridax.nzt
Temperature of the holder	:	25.1 ℃
Viscosity of the dispersion medium	:	0.893 mPa·s
Conductivity	:	0.088 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-41.7 mV	-0.000324 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-41.7 mV
Electrophoretic Mobility mean	:	-0.000324 cm^2/Vs

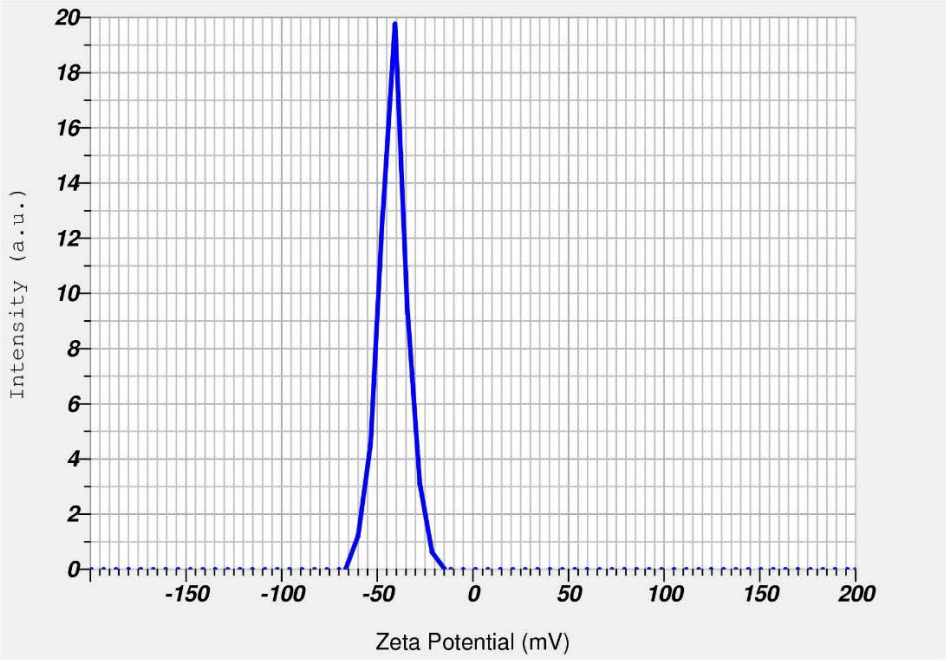


Figure 6. Zeta Potential analysis of *E. hirta* mediated green synthesized of AgNPs

Measurement Results

Euphorbia.nzt

Measurement Results

Date	:	Tuesday, December 17, 2014 3:01:52 PM
Measurement Type	:	Zeta Potential
Sample Name	:	Euphorbia
Temperature of the holder	:	25.1 ℃
Viscosity of the dispersion medium	:	0.893 mPa·s
Conductivity	:	0.070 mS/cm
Electrode Voltage	:	3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-27.9 mV	-0.000216 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-27.9 mV
Electrophoretic Mobility mean	:	-0.000216 cm^2/Vs

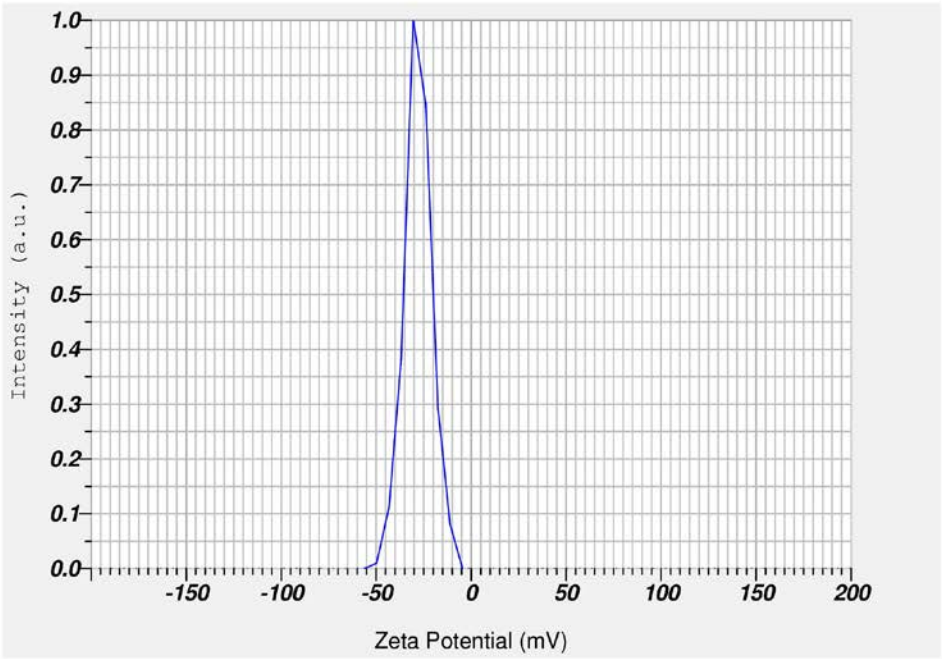


Figure 7. Zeta Potential analysis of green synthesized AgNPs using *A. indica*

Measurement Results

Neem.nzt

Measurement Results

Date	: Tuesday, December 17, 2014 3:43:19 PM
Measurement Type	: Zeta Potential
Sample Name	: Neem
Temperature of the holder	: 25.2 ℃
Viscosity of the dispersion medium	: 0.891 mPa·s
Conductivity	: 0.066 mS/cm
Electrode Voltage	: 3.9 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-37.2 mV	-0.000290 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	: -37.2 mV
Electrophoretic Mobility mean	: -0.000290 cm^2/Vs