2. REVIEW OF LITERATURE

2.1. NANOTECHNOLOGY

Nanotechnology is a multidisciplinary field, as it combines the knowledge from different disciplines: chemistry, physics, and biology amongst others (Schmid, 2006; Schmid, 2010). Nanotechnology is the art and science of manipulating matter at the atomic or molecular scale and holds the promise of providing significant improvements in technologies for protecting the environment. While many definitions for nanotechnology exist, the U.S. Environmental Protection Agency (EPA) uses the definition developed by the National Nanotechnology Initiative (NNI). According to National Nanotechnology Initiative of the USA, nanotechnology is defined as: research and technology development at the atomic, molecular, or macromolecular levels using a length scale of approximately one to one hundred nm in any dimension; the creation and use of structures, devices and systems that have novel properties and functions because of their small size; and the ability to control or manipulate matter on an atomic scale (USEPA, 2007). The technology has excellent prospects for exploitation across the medical, pharmaceutical, biotechnology, engineering, manufacturing, telecommunications and information technology markets.

2.2. HISTORY AND DEVELOPMENT OF NANOTECHNOLOGY

It is difficult to describe the history of nanotechnology which, according to R. D. Booker is due to two principal reasons: (1) Ambiguity of the term “nanotechnology” and (2) Uncertainty of the time span corresponding to the early stages of nanotechnology development. The term nanotechnology is explained by a wide spectrum of various technologies that nanotechnology covers, which are based on various types of physical, chemical and biological processes, realized at nanolevel.

The strictly established time span for the beginning of nanotechnology development is explained by the fact that nanotechnology has its backgrounds in the distant past when people used it without knowledge of it (Tolochko). The difference between these ancient examples of “nanotechnology” and the current situation is the
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ability to understand or at least embark on a path towards understanding—the fundamental principles underlying nanotechnological behavior, the ability to assess the current state of knowledge, and the ability to systematically plan for the future based on that knowledge (USEPA, 2007).

The word “nanotechnology” was introduced for the first time by Norio Taniguchi at the International Conference on Industrial Production in Tokyo in 1974 in order to describe the super thin processing of materials with nanometer accuracy and the creation of nano-sized mechanisms. Ideas of nanotechnological strategy, which were put forward by Richard Feynman (known as “Father of Nanotechnology”) in his lecture delivered in 1959 at the session of the American Physical Society, were developed by Eric Drexler in 1986. Nanotechnology and nanoscience got a boost in the early 1980s with two major developments: the birth of cluster science and the invention of the Scanning Tunneling Microscope (STM) in 1981. These developments led to the discovery of Fullerenes in 1985 and the structural assignment of Carbon Nanotubes in 1991.

In the second half of 1980s and early 1990s a number of important discoveries were made, this created an essential impact on the further development of nanotechnology. In 1991, the first nanotechnological program of National Scientific Fund started to operate in USA. In 2001, the National Nanotechnological Initiative (NNI) of the USA was approved. Since then, lots of scientific and technical research developments have been taking place all over the world especially in countries like Japan, Germany, England, France, China, South Korea and recently in the CIS countries.

Thus, the nanotechnology paradigm was formed at the turn of the 1960s, while the 1980s and 1990s are the start of development of nanotechnology in its own right. Hence the whole period up to the 1950s may be considered as pre-history of nanotechnology. The end of this period was the appearance of conditions for managed nanotechnology development, which was facilitated by scientific and technical revolution (Tolochko). It is now widely accepted that nanotechnology is emerging as a major factor for commercial success in the 21st century and is regarded as “the next industrial revolution”.
2.3. **NANOPARTICLES**

Nanotechnology is getting developed at several levels: materials, devices and systems. The nanomaterials level is the most advanced at present, both in scientific knowledge and in commercial applications (Salata, 2004). Nanoparticles are less than a few 100 nm. This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. They can be metallic, mineral, polymer-based or a combination of materials (Rana and Kalaichelvan, 2013). Most of these changes are related to the appearance of quantum effects as the size decreases, and are the origin of phenomena such as the superparamagnetism, Coulomb blockade, surface plasmon resonance, etc. The increase in the surface area to volume ratio is also a consequence of the reduction in size. It leads to the appearance of surface effects related to the high number of surface atoms, as well as to a high specific area, which are important from the practical point of view (Salata, 2004).

### 2.4. PROPERTIES OF NANOPARTICLES

Once the particle size is reduced below 100 nm, the solid particles begin to demonstrate unusual properties from the bulk material based on Quantum mechanics (Bhusan, 2007). The surface related properties and the quantum properties play a fundamental role in making the difference in the properties of the bulk material with that of the nanoparticles (Roduner, 2006). They exhibit-

**Size effects** - Depending on the material used to produce nanoparticles, properties like solubility, transparency, color, absorption or emission wavelength, conductivity, melting point and catalytic behavior are changed only by varying the particle size.

**Surface effects** - Properties like dispersibility, conductivity, catalytic behavior and optical properties alter with different surface properties of the particle.

If the surface properties are not controlled, nanoparticles quickly turn into larger particles due to agglomeration. Most of the size dependent effects are then lost. For the application of nanoparticles, it is therefore crucial to control their agglomeration behavior. Dispersed nanoparticles are needed in order to retain their specific properties for the technological applications as shown in (Plate 1) (Borm et al., 2006).

The nanoparticles exhibit various unique features like optical, structural, thermal, mechanical and electromagnetic properties as described below.
2.4.1. Optical properties

As the size of particles size is reduced to nanometer level, depending upon the kind of metal nanoparticles and particle size, they absorb the light with a specific wavelength and due to Surface Plasmon Resonance i.e., the interaction of electromagnetic radiation and the electrons in the conduction band around the nanoparticles (Mulvaney, 1996; Park and Kim, 2008) they transmit different colors. It is possible to perform quantitative and qualitative analyses of particle size and distributions and particle concentration and the effect of particle shape as consequence of optical spectrum shifts.

The gold and silver nanoparticles show the color phenomena with splendid tinting strength, color saturation and transparency. For instance, bulk gold appears yellow in color, but nanosized gold appears red in color. Furthermore, since the nanoparticles are smaller than the wavelength of visible light and the light scattering by the particles becomes negligible, higher transparency can be obtained with the nanoparticles than the conventional pigment. As seen in the Raman Effect phenomena, decreasing size of the particle will increase the Kubo gap and therefore the energy emitted by the photons will change frequency and hence their color (Nobile et al., 2007; Xu and Cortie, 2006).

2.4.2. Structural properties

The large specific surface area of the nanoparticles is an important property related to reactivity, solubility, sintering performance etc. and is also related with the mass and heat transfer between the particles and their surroundings. Furthermore, the crystal structure of the particles may change with the particle size in the nanosized range in many cases. This is attributed to the compressive force exerted on the particles as a result of the surface tension of the particle itself. The critical particle size of the crystal structure and the size effect differ with the materials.

2.4.3. Thermal properties

As the atoms and molecules located at the particle surface become significant in the nanometer order, the melting point of the material decreases from that of the bulk material because they tend to move easier at the lower temperature. The reduction of the melting point of ultrafine particles is regarded as one of the unique features of the nanoparticles related with aggregation and grain growth of the nanoparticles or
improvement of sintering performance of ceramic materials. Hence, melting point of nanomaterials differs from their corresponding bulk materials as an end result of their free surface and size. Several examples could be found to illustrate the melting point depression as function of the particle size. (Shrivastava, 2002).

2.4.4. Mechanical properties

The hardness of crystalline materials increases with the decreasing crystalline size, and that the mechanical strength of the materials considerably increases by micronizing the structure of the metal and ceramic material or composing them in the nano range (Niihara, 1991).

2.4.5. Electromagnetic properties

When the materials are reduced to the nanolevel, electromagnetic forces become predominant in these nanoparticles. The mass of the nanoscale object is so small, that the gravity becomes negligible and electromagnetic forces overtake the gravitational force. The nanoparticles are raw materials for a number of electronic devices. The electromagnetic properties play a great role for the improvement of the product performance. The minimum particles size to keep the ferroelectric property differs depending upon the kind and composition of the materials.

As for the magnetic property, ferromagnetic fine particles have a single magnetic domain structure as they become very small as in the order less than about 1 μm and show super-paramagnetic property, when they get further finer. In this case, although the individual particles are ferromagnetic with the single magnetic domain structure, the particles collectively behave as paramagnetic. It is magnetized as a whole in the same direction of the external magnetic field but the magnetization disappears by the thermal fluctuation, when the external magnetic field is taken away. The time for disappearing of magnetization depends upon the particle size, like, the magnetization of the material responds with the external magnetic field as a paramagnetic when the particles are small enough but it decreases gradually as the particle size becomes larger. Gold which is a stable substance as bulk shows unique catalytic characteristics as nanoparticles (Matsui, 2005).
2.5. APPLICATIONS OF NANOPARTICLES

The nanotechnology market can be broadly divided into 3 segments, viz. Materials, Tools and Devices:

**Nanomaterials** – used to describe materials with one or more components that have at least one dimension in the range of 1 to 100 nm and include Nanoparticles, nanofibres and nanotubes, composite materials and nano-structured surfaces. These include Nanoparticles (NP) as a subset of nanomaterials currently defined by consensus as single particles with a diameter < 100 nm. Agglomerates of NP can be larger than 100 nm in diameter but will be included in the discussion since they may break down on weak mechanical forces or in solvents. Nanofibres are a sub-class of nanoparticles (include nanotubes) which have two dimensions < 100 nm but the third (axial) dimension can be much larger.

**Nanotools** – tools and techniques for synthesizing nanomaterials, manipulating atoms and fabricating device structures, and very importantly for measuring and characterizing materials and devices at the nanoscale;

**Nanodevices** – making devices at the nanoscale, important in microelectronics and optoelectronics at the present time, and at the interface with biotechnology where the aim is to mimic the action of biological systems such as cellular motors. This latter area is the most futuristic, and excites the greatest public reaction (Borm et al., 2006).

2.5.1. Approaches to make nanotechnology products

Nanotechnology brings out the idea that the assembly can be hierarchical and controlled in specific ways. There are two distinct approaches to making products with nanoscale features and attributes:

**Top-down fabrication** is the method used in the microelectronics industry, where small features are created on large substrates by repeated pattern transfer steps involving lithographic methods. Extreme UV photolithography can produce patterns with feature sizes down to 100 nm, and electron beam lithography can be used for features down to 30 nm.

**Bottom-up fabrication** is directly relevant to the chemicals industry. This method starts with very small units, often individual molecules or even atoms, and assembles
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these building-block units into larger structures clearly the domain of chemistry (Borm et al., 2006).

2.5.2. Applications of nanoparticles in Therapeutics, health and medicine

A confluence of nanotechnology and biology can address several biomedical problems, and can revolutionize the field of health and medicine (Curtis and Wilkinson, 2001). Nanotechnology is currently employed as a tool to explore the darkest avenues of medical sciences in several ways like imaging (Waren and Nie, 1998) sensing (Vaseashta and Malinovska, 2005), targeted drug delivery (Langer, 2001) and gene delivery systems (Roy et al., 1999) and artificial implants (Sachlos et al., 2006). Hence, nanosized organic and inorganic particles are finding increasing attention in medical applications (Xu et al., 2006) due to their amenability to biological functionalization. Based on enhanced effectiveness, the new age drugs are nanoparticles of polymers, metals or ceramics, which can combat conditions like cancer (Farokhzad et al., 2006) and fight human pathogens like bacteria (Stoimenov et al., 2002; Sondi and Sondi, 2004; Panacek et al., 2006; Morones et al., 2005; Baker et al., 2005).

Many nanoparticles like silver are useful as therapeutics due to their antimicrobial properties. Polyisohexylcyanoacrylate nanoparticles, poly(lactic-co-glycolic acid) (PLGA) nanoparticles, Gold nanoparticles, Chitosan nanoparticles, Cetyl alcohol/polysorbate nanoparticles, Lipid nanocapsules, P (4-vinylpyridine) particles, Chitosan-alginate nanoparticles, Poly (3-hydroxybutyrate-co-3 hydroxyoctanoate) nanoparticles are some of the nanoparticles that can be effectively used for therapeutics (Barraud et al., 2005; Cheng et al., 2010; Chithrani et al., 2010; Hee-Dong et al., 2010; Koziara et al., 2004; Lamprecht et al., 2006; Ozay et al., 2010; Parveen et al., 2010; Zhang et al., 2010).

Nanoparticles has also been modified for early detection of Alzheimer's disease biomarkers in biological fluids as well as delivery of bioactive molecules directly to brain. Although nanotechnology is expected to have a huge impact on the development of “smart” drug delivery and devices against Alzheimer's disease, a crucial gap still to be filled concerns the elucidation of its etiology, for which a great deal of effort is required (Brambilla et al., 2011).
2.5.3. Applications of nanoparticles as Biosensors

Nanoparticles (NPs), with colorful light-scattering properties, have unique advantages and are comparable to optical probes with various fluorescent dyes. Metal nanoparticles with large diameter (>30 nm) exhibit strong light scattering in the visible region. This can be used directly for light scattering labels in biochemical assay. Nanosensors, capable of providing data through unique technology, could find wide application in monitoring our personal health, the food we eat, and our environmental health. The performances of nano biochemical sensors are excellent in terms of sensitivity, selectivity, linearity, stability, response time, and reproducibility compared to the traditional biosensors. The nanoparticles labeling procedure is very simple, and the biochemical activity of the labeled compound is almost unaffected. This new approach is critically useful in preventing interference between chemically related analytes. Many nanosensors have been developed like glucose nanosensors (Wang et al., 2009), choline nanosensors (Wang and Musamaeh, 2003), Nicotinamide adenine dinucleotide nanosensors (Gopalan et al., 2009), Lactate nanosensors (Parra et al., 2006), Triglyceride nanosensors (Vijayalakshmi et al., 2008), Urea nanosensors (Seo et al., 1993). Nanoparticles have also been developed in bioassays for detection of Human immunoglobulin G (Cui et al., 2001), Steroids (Macara and Lannigan, 2005) etc.

2.5.4. Applications of nanoparticles in Food industries

In food industry, several novel applications of nanotechnologies have become apparent, including the use of nanoparticles, such as micelles, liposomes, nanoemulsions, biopolymeric nanoparticles and cubosomes, as well as the development of nanosensors, which are aimed at ensuring food safety (Yih and Al-Fandi, 2006; Nasongkla et al., 2006; Esposito et al., 2005; Ligler et al., 2003). Some examples of the use of nanotechnology in food products are cooking oils that contain nutraceuticals within nanocapsules, nanoencapsulated flavor enhancers and nanoparticles that have the ability to selectively bind and remove chemicals from food.

2.5.5. Other industrial applications

2.5.5.1. Textile industries

The novel properties and low material consumption amount has attracted global interest across disciplines and industries. The textile sector is no exception. Health
concerns along with customer satisfaction have made functionally finished fabric a fast-paced and fast growing industry. It soon became more important for antimicrobial finished fabrics to protect the wearer from bacteria than it was to simply protect the garment from fiber degradation (Yadav et al., 2006). The need for antimicrobial fabrics goes hand-in-hand with the rise in resistant strains of microorganisms. Functional textiles include everything from antimicrobial finished textiles, to durable, or permanent press finished garments, to textiles with self-cleaning properties, and also textiles with nanotechnology (Rajendran et al., 2010; El-Rafie et al., 2010). Coated antimicrobial sutures have also been developed to aid fast would heal without microbial infection (Dubas et al., 2011).

2.5.5.2. Dye and paint industries

Paint industries have also utilized the properties of nanoparticles. The appearance and usefulness of nanoparticles brings many advantages and opportunities to paint and coating industry. Addition of nanoparticles to coatings can upgrade many properties of coating system and can produce multipurpose coatings with a little cost difference. They have lots of advantages like, better surface appearance, good chemical resistance, decrease in permeability to corrosive environment and hence better corrosion properties, optical clarity, increase in modulus and thermal stability, easy to clean surface, anti-skid, anti-fogging, anti-fouling and anti-graffiti properties, better thermal and electrical conductivity, better retention of gloss and other mechanical properties like scratch resistance, anti-reflective in nature, chromate and lead free, good adherence on different type of materials (Khanna, 2008). Nanoparticles also aid in dye degradation and it has been found that AgNPs are good, highly efficient and stable photocatalysts under ambient temperature with visible light illumination for degrading organic compounds and dyes (Wang et al., 2008).

2.5.5.3. Wastewater treatment

In the area of water purification, nanotechnology offers the possibility of an efficient removal of pollutants and germs. Today nanoparticles, nanomembrane and nanopowder used for detection and removal of chemical and biological substances include metals (e.g. Cadmium, copper, lead, mercury, nickel, zinc), nutrients (e.g. Phosphate, ammonia, nitrate and nitrite), cyanide, organics, algae (e.g. cyanobacterial toxins) viruses, bacteria,
parasites and antibiotics. Basically four classes of nanoscale materials are being evaluated as functional materials for water purification i.e., metal-containing nanoparticles, carbonaceous nanomaterials, zeolites and dendrimers. Carbon nanotubes and nanofibers also show some positive result. Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio) (Tiwari et al., 2008).

2.5.5.4. Energy/ Fuel cells/ Solar cells

Efficient production of solar fuels is an imperative for meeting future fossil-fuel-free energy demands. Spencer et al., 2010 grafted light-harvesting molecules (polymer coated gold nanoparticles where inorganic catalysts or light-harvesting porphyrin molecules) to nanoparticles surfaces to make nanocells for the generation of fuel. Nanoparticles when added to heat transfer fluids increase their performance. The solid nanoparticles conduct heat better than the liquid. Nanoparticles work best because they stay suspended in liquids longer than larger particles. They also have a much greater surface area, which is where heat transfer takes place. The smaller the particle, the greater is its ability to enhance heat transfer. Nano-additives, including nanoparticles and nanopowder, could be utilized to enhance heat transfer from solar collectors to storage tanks. Nanocrystalline nickel and metal hydrides have been investigated for use in batteries. The nanostructured materials offer improvements regarding power density and durability by controlling the charge diffusion and oxidation state on a nanoscale level. Magnetic fluids, which have significant heat transfer capabilities when exposed to magnetic fields, are being investigated as substitutes for existing coolants in transformers for electrical supply (Strem Chemicals Inc., 2013).

2.6. METAL NANOPARTICLES

Metal nanoparticles are nanoparticles of metals like gold, silver, iron copper etc. They are a focus of interest because of their huge potential in nanotechnology. Today these materials can be synthesized and modified with various chemical functional groups which allow them to be conjugated with antibodies, ligands, and drugs of interest and thus opening a wide range of potential applications in biotechnology, magnetic separation, and pre-concentration of target analytes, targeted drug delivery, and vehicles for gene and drug delivery and more importantly diagnostic imaging (Mody et al., 2010).
2.7. SILVER NANOPARTICLES

Silver nanoparticles (AgNPs) are particles of silver that range from 1 to 100 nm. While frequently described as being 'silver' some are composed of a large percentage of silver oxide due to their large ratio of surface to bulk silver atoms. Currently, there is also an effort to incorporate AgNPs into a wide range of medical devices, including bone cement, surgical instruments, surgical masks, etc.

Moreover, it has also been shown that ionic silver, in the right quantities, is suitable in treating wounds (Qin, 2005; Atiyeh et al., 2007; Lansdown, 2006). In fact, AgNPs are now replacing silver sulfadiazine as an effective agent in the treatment of wounds. Additionally, Samsung has created and marketed a material called Silver Nano, which includes AgNPs on the surfaces of household appliances. Moreover, due to their attractive physiochemical properties these nanomaterials have received considerable attention in biomedical imaging using SERS. In fact, the surface plasmon resonance and large effective scattering cross-section of individual AgNPs make them ideal candidates for molecular labeling (Schultz et al., 2000). Thus many targeted silver oxide nanoprobe are currently being developed (Mody et al., 2010).

2.7.1. Why silver nanoparticles?

One of the first and most natural questions to ask when starting to deal with silver nanoparticles (AgNPs) is: “Why are AgNPs so interesting”? Why even bother to work with these extremely small structures when handling and synthesis is much more complicated than that of their macroscopic counterparts. The answer lies in the nature of and unique properties possessed by nanostructures. AgNPs possess a very high surface to volume ratio. This can be utilized in areas where high surface areas are critical for success. This could for example be in the catalytic industry; some nanoparticles actually have proven to be good catalysts. Some AgNPs also show bactericidal effects and here a high surface to volume ratio is also important. In biology and biochemistry AgNPs have attracted much attention.

AgNPs are often in the range 1-100 nm and this is the size as that of human proteins. AgNPs exhibit many interesting properties (Bozhevolnyi et al., 2006). When a silver particle decreases in the order of one millionth of a millimeter, at this state, the fundamental physical property of the material can change drastically and materials may
be sintered at a lower temperature. Also, as particles get smaller than the wavelength of visible light, they not only become transparent but also emit special light by plasma absorption. They show completely different electromagnetic or physicochemical properties from their bulk counterparts, although they are made of the same materials.

AgNPs have attracted the attention of researchers because of their unique properties, and proven applicability in diverse areas such as medicine, catalysis, textile engineering, biotechnology, nanobiotechnology, bioengineering sciences, electronics, optics, and water treatment. Moreover, AgNPs have significant inhibitory effects against microbial pathogens, and are widely used as antimicrobial agents in a diverse range of consumer products, including air sanitizer sprays, socks, pillows, slippers, respirators, wet wipes, cosmetics, detergents, soaps, shampoo, toothpastes, air and water filters, coatings of refrigerators, vacuum cleaners, bone cement, wound dressings, surgical dressings, washing machines, food storage packaging, and cell phones. The flexibility of silver nanoparticle synthetic methods and facile incorporation of AgNPs into different media have interested researchers to further investigate the mechanistic aspects of antimicrobial, antiviral and anti-inflammatory effects of these nanoparticles (Korbekandi and Iravani, 2012).

### 2.7.2. Properties and Applications

AgNPs exhibit various properties and have been intensely used in various applications. Given below are a important ones described in brief.

#### 2.7.2.1. Antimicrobial activity

Silver is a metal known for its broad spectrum antimicrobial activity against Gram positive and Gram negative bacteria, fungi, protozoa and certain viruses. The persistence of antibiotic resistant bacteria has exploited the antimicrobial properties of silver and silver-based compounds, including AgNPs (Nair and Laurencin, 2007). The mechanism of cellular toxicity exhibited by metal nanoparticles is through the release of Reactive Oxygen Species (ROS) (Nel et al., 2009). The antibacterial properties of AgNPs are associated with its slow oxidation and liberation of Ag\(^+\) ions to the environment making it an ideal biocidal agent. Moreover, the small size of these particles facilitates the penetration of these particles through cell membranes to affect intracellular processes from inside.
Additionally, the excellent antibacterial properties exhibited by the nanoparticles are due to their well developed surface which provides maximum contact with the environment (Krutyakov et al., 2008). A better understanding of the bactericidal action of nanosilver would require a proper examination of the membrane-bound and intracellular nanoparticles. AgNPs were found to penetrate into the bacterial cell causing membrane damage and ultimately the death of the organism. Due to this reason, AgNPs are being used in coating antimicrobial fabrics, sutures etc. According to the reports of Kim et al., 2009, AgNPs exhibited excellent antifungal activity on Candida albicans by disrupting the cell membrane and inhibiting the normal budding process (Kim et al., 2009).

2.7.2.2. Optical properties

AgNPs are known for their excellent optical properties. These unique optical properties originate from the collective oscillations of conduction electrons termed as surface plasmon resonance. Size-dependent localized surface plasmon resonance contributes to surface enhanced Raman signals intense enough to detect single molecules.

2.7.2.3. As catalyst

A possible application of AgNPs is the use as a catalyst. AgNPs immobilized on silica spheres have been tested for their ability to catalyze the reduction of dyes by sodium borohydride (NaBH₄). Catalysis of dyes was chosen because it is easy to detect a change in color when the dyes are reduced. In the absence of AgNPs the sample was almost stationary showing very little or no reduction of the dyes (Jiang et al 2004) (Bozhevolnyi et al., 2006).

2.7.2.4. As biosensor

Optical sensors of zeptomole (10⁻²¹) sensitivity are another possible application using the potential of AgNPs. Using the surface plasmon resonance effect the AgNPs gain a very high sensitivity and the measurements can be conducted in real-time. AgNPs show a peak in extinction, due to the localized surface plasmon resonance (LSPR) effect. More precisely this is caused by a collective excitation of the conduction band electrons of the nanoparticles (McFarland and Duyne, 2003) (Bozhevolnyi et al., 2006).

2.7.2.5. Anti-inflammatory activity

AgNPs have wound healing properties as was demonstrated by Tian et al. (2007) who found that rapid healing and improved cosmetic appearance occurred in a dose-
dependent manner by the topical delivery of AgNPs. These NPs also exert positive effects through their antimicrobial properties, reduction in wound inflammation and modulation of fibrogenic cytokines.

2.7.3. Synthesis of silver nanoparticles

There are a large variety of methods that can be used to in the production of nanoparticles, making use of techniques from both physics and chemistry. Among the first ones, arc-discharge, high-energy ball milling, laser pyrolysis and laser ablation are the most commonly used. Electrochemical and chemical vapor deposition, sonochemistry and different wet chemistry routes (e.g. sol-gel, co-precipitation, inverse micelles, etc.) are also widely employed (Melendi et al., 2008). With the development of new chemical or physical methods, the concern for environmental contaminations is also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous byproducts. Thus, there is a need for ‘green chemistry’ that includes a clean, nontoxic and environment-friendly method of nanoparticle synthesis (Sharma et al., 2007; Mukherjee et al., 2001). As an alternative to conventional methods, biological methods are considered safe and ecologically sound for the nanomaterial fabrication (Shankar et al., 2004a).

2.8. BIOSYNTHESIS OF SILVER NANOPARTICLES

In the chemically synthesized metal nanoparticles, the toxic chemicals and strong reducing agents like sodium citrate which are involved in the synthesis process and byproducts formed during the synthesis play a major role in producing the cytotoxic effect (Seon et al., 2012). Capping agents or the stabilizing agents are reducing agents like sodium citrate which gives more negative surface charge to the nanoparticles. This negative surface charge also plays a pivotal role in the toxic effect of the chemically synthesized gold nanoparticles (Seon et al., 2012). When the biologically synthesized nanoparticles are considered and compared with that of chemically synthesized nanoparticles, the cytotoxic effect is more for chemically synthesized nanoparticles (Girilal, 2013). The least toxicity was observed for biologically synthesized silver nanoparticles as it is a pure green synthesis method which does not involve the use of any other toxic chemicals except corresponding metal halides like silver nitrate. Proteins and enzymes play a major role in the biological synthesis process and these proteins also act
as capping or stabilizing agents to the nanoparticles instead of the toxic chemicals or reducing agents as in the case of chemically synthesized metal nanoparticles (Ahmad et al., 2003). This might have contributed to the lesser toxicity levels of biologically synthesized nanoparticles when compared to the chemically synthesized ones.

Biosynthesis of AgNPs has been done using bacteria, fungi, actinomycetes, yeasts, algae and plants have been found to be capable of intracellularly or extracellularly synthesizing nanoparticles, mineral crystals and metallic nanoparticles. Nanoparticle synthesis with bacteria and fungi has gained more interest compared to actinomycetes and yeasts because of the well established technology available in synthesis by bacteria and fungi than by actinomycetes, yeasts and algae. Some well-known examples of microbial systems synthesizing inorganic materials include magnetotactic bacteria for magnetite nanoparticles (Spring and Schleifer, 1995; Dickson, 1999), S-layer bacteria for gypsum and calcium carbonate layers (Pum and Sleytr, 1999) and silver mine-inhabiting Pseudomonas sp. that reduces silver ions to form AgNPs (Joerger et al., 2000). Nanocrystals of gold, silver and their alloys have been synthesized within the cells of lactic acid bacteria (Nair and Pradeep, 2002). Fungus and actinomycete species were reported to synthesize silver or gold nanoparticles of different shapes and sizes (Shankar et al., 2004b; Ahmad et al., 2003).

Over the last few decades, it was only the prokaryotes that have been exploited for the capability to biosorb and bioreduce insoluble toxic metal ions to soluble non-toxic metal salts or change in valency. But recently, it was found that highly evolved organisms like fungi, plants, algae, diatoms and even human cells possess the reducing potential to convert the inorganic metal ions to metal nanoparticles. Eukaryotes have more information in their genetic material to encode various reducing/stabilizing agents that mediate the synthesis of metal nanoparticles. Phototrophic eukaryotes acquire energy from sunlight through photosynthesis and fixes inorganic carbon into organic materials, whereas heterotrophic eukaryotes like human cells use organic carbon produced by other organisms for growth. Terrestrial environment contains predominant phototrophs as plants, algae and diatoms in aquatic environment.

Exploration of the plant systems as the potential nanofactories has heightened interest in the biological synthesis of AgNPs (Krishnaraj et al., 2010). Plant mediated
nanoparticles synthesis is getting more popular because of the high reactivity of plant extract and easy availability of plant materials. In a comparative in-vitro and in-vivo toxicity study carried out by Girilal (2013), it has been concluded that biologically synthesized AgNPs are less toxic to the chemically synthesized AgNPs. This method of nanoparticles synthesis involves no toxic chemicals and has been termed as Green chemistry procedure (Krishnaraj et al., 2012). An overview of various reports on AgNPs synthesis by different plants is presented in (Table 1).

2.8.1. Mechanism of synthesis of biogenic silver nanoparticles

Synthesis of AgNPs using plant extracts is getting more popular (Li et al., 2007; Song and Kim, 2009). Chandran et al. synthesized AgNPs by using the Aloe vera extract at 24 h of incubation (Chandran et al., 2006). Similar study was carried out by Krishnaraj et al., 2010 using leaf extracts of Acalypha indica. Interestingly, AgNPs were synthesized rapidly within 30 min of incubation period. The aqueous silver nitrate solution was turned to brown color within 30 min, with the addition of leaf extract. Intensity of brown color increased in direct proportion to the incubation period (Krishnaraj et al., 2010).

The possible mechanism of biosynthesis of nanoparticles by biological system is reductases and any other equivalent reductants as reported earlier (Shankar et al., 2004a). The nitrate reductase from Fusarium oxysporum has been documented to catalyze the reduction of AgNO$_3$ to AgNPs utilizing NADPH as reducing agent Duran et al., 2005). Several naphthoquinones and anthraquinones having very high redox potentials have been reported from F. oxysporum that could act as an electron shuttle in metal reduction (Newman and Kolter, 2000). Although such systems were not repeated in plant mediated synthesis nanoparticles, the phytochemical constituents are attributed to the formation of nanoparticles. Caffeine and theophylline present in tea extracts were also reported to catalyze the synthesis of nanoparticles (Groning et al., 2001). Phyllanthin from Phyllanthus amarus was also reported as the capping ligands in the synthesis of AgNPs (Kasthuri et al., 2009). Quercetin and polysaccharides have been used for silver nanoparticle synthesis (Egorova et al., 2000).

2.9. CHARACTERIZATION OF NANOPARTICLES

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

2.9.1. UV-Vis spectroscopy

The electronic structures of atoms, ions, molecules or crystals through exciting electrons from the ground to excited states (absorption) and relaxing from the excited to ground states (emission) are used for determination in UV-Vis spectroscopy. It deals with the study of electronic transitions between orbitals or bands of atoms, ions or molecules in gaseous, liquid and solid state (Jorgensen, 1962). Small metallic nanoparticles are proven to have the property for absorption and scattering electromagnetic radiation. The metallic nanoparticles are also known to exhibit different characteristic colors. The absorption of electromagnetic radiation by metallic nanoparticles originates from the coherent oscillation of the valence band electrons induced by an interaction with the electromagnetic field (Faraday, 1857). These resonances are known as surface plasmons, which occur only in the case of nanoparticles and not in the case of bulk metallic particles (Papavassiliou, 1979). Hence, UV-Vis spectroscopy can be utilized to study the unique optical properties of nanoparticles (Link and El-sayed, 1999; Burda et al., 2000).

2.9.2. Fourier Transform Infrared (FT-IR) spectroscopy

In Fourier transform infrared spectroscopy, the vibrational technique involved is the interactions of photons with species in a sample that results in energy transfer to or from the sample via vibrational excitation or de-excitation is exploited for characterization. These vibrational frequencies provide the information of chemical bonds in the detecting samples. It deals with the vibration of chemical bonds in a molecule at various frequencies depending on the elements and types of bonds. After absorbing electromagnetic radiation the frequency of vibration of a bond increases leading to transition between ground state and several excited states. These absorption frequencies represent excitations of vibrations of the chemical bonds and thus are specific to the type of bond and the group of atoms involved in the vibration. The energy corresponding to these frequencies correspond to the infrared region of the electromagnetic spectrum. The FT-IR measurement can be utilized to study the presence of protein molecule in the solution, as the FT-IR spectra in the 1400 cm\(^{-1}\)-1700 cm\(^{-1}\)
region provides information about the presence of –CO- and –NH- groups (Banwell and McCash, 1996).

2.9.3. X-ray diffraction (XRD)

X-ray diffraction has been used to determine the crystal structure of solids, including lattice constants and geometry, identification of unknown materials, orientation of single crystals, defects, etc (Wang, 2000). The X-ray diffraction patterns are obtained by measurement of the angles at which an X-ray beam is diffracted by the crystalline phases in the specimen.

Bragg’s equation relates the distance between two hkl planes (d) and the angle of diffraction (2θ) as:

\[ n\lambda = 2d\sin\theta, \]

where,

\[ \lambda = \text{wavelength of X-rays}, \]
\[ n = \text{an integer known as the order of reflection} \]

\[ (h, k \text{ and } l \text{ represent Miller indices of the respective planes}) \] (Bragg and Bragg, 1949).

From the diffraction patterns, the uniqueness of nanocrystal structure, phase purity, degree of crystallinity and unit cell parameters of the Nanocrystalline materials can be determined.

X-ray diffraction technique is nondestructive and does not require elaborate sample preparation, which partly explains the wide use of XRD methods in material characterization. X-ray diffraction broadening analysis has been widely used to determine the crystal size of nanoscale materials. The average size of the nanoparticles can be estimated using the Debye–Scherrer equation:

\[ D = k\lambda / \beta\cos\theta \]

where

\[ D = \text{thickness of the nanocrystal}, \]
\[ k \text{ is a constant}, \]
\[ \lambda = \text{wavelength of X-rays}, \]
\[ \beta = \text{width at half maxima of } (111) \text{ reflection at Bragg’s angle } 2\theta \] (Rau, 1962; Birks and Friedman 1946).
2.9.4. Transmission electron microscopy (TEM)

Electron microscopes are scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. The transmission electron microscope (TEM) was the first type of Electron Microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to "see through" the specimen. It was developed by Max Knoll and Ernst Ruska in Germany in 1931. Transmission electron microscopy is typically used for high resolution imaging of thin films of a solid sample for nanostructural and compositional analysis. The topographic information obtained by TEM in the vicinity of atomic resolution can be utilized for structural characterization and identification of various phases of nanomaterials (Wang, 2000). The technique involves: (i) irradiation of a very thin sample by a high-energy electron beam, which is diffracted by the lattices of a crystalline or semi crystalline material and propagated along different directions, (ii) imaging and angular distribution analysis of the forward-scattered electrons (unlike SEM where backscattered electrons are detected), and (iii) energy analysis of the emitted X-rays (Fryer, 1979).

2.10. RESEARCH IN THE CURRENT THESIS

2.10.1. Synthesis of biogenic silver nanoparticles using plant extract

In biological synthetic methods, it has been shown that the AgNPs produced by plants are more stable in comparison with those produced by other organisms. Plants (especially plant extracts) are able to reduce silver ions faster than fungi or bacteria. Furthermore, in order to use easy and safe green methods in scale-up and industrial production of well-dispersed AgNPs, plant extracts are certainly better than plant biomass or living plants (Korbekandi and Iravani, 2012). Hence in the current research work, biosynthesis of AgNPs was carried out using rhizomes of Alpinia calcarata Rosc. as there are no records of research work done in AgNP synthesis using this plant.

*Alpinia calcarata* Rosc. (Zingiberaceae) is a medicinal plant and is used for several pharmaceutical purposes. It is cultivated in tropical countries including Sri Lanka, India and Malaysia. The rhizomes of *Alpinia calcarata* Rosc. are used for medicinal purposes (Jayaweera, 1982). Extracts of *Alpinia calcarata* Rosc. have been found to show antibacterial (George and Pandalai, 1949) antifungal (Pushpangadan and Atal,
1984) and antihelminthic activity (Kaleysa, 1974). The extracts of rhizomes have also been used in the treatment of bronchitis, cough, respiratory ailments, diabetics, asthma (Ramanayake and Visithuru, 1994) and arthritis (Ramanayake and Visithuru, 1994; Arambewela et al., 1995). The rhizomes contain phytochemicals like polyphenols, tannins, flavonoids, alkaloids, steroid glycosides that may act as reducing and stabilizing agent in the green synthesis of biogenic AgNPs (Arambewela LSR and Arawwawala, 2010; Krishnaraj et al., 2010).

2.10.2. Antibacterial and antifungal activities of biogenic silver nanoparticles

Metal nanoparticles with bactericidal activity can be immobilized and coated on to surfaces, which may find application in various fields, i.e., medical instruments and devices, water treatment and food processing. Metal nanoparticles may be combined with polymers to form composites for better utilization of their antimicrobial activity. The antimicrobial properties of AgNPs are well-established (Sondi and Sondi, 2004; Kumar et al., 2005; Jain and Pradeep, 2005; Cho et al., 2005) and several mechanisms for their bactericidal effects have been proposed. The antimicrobial effects of metal nanoparticles are attributed to their small size. Antimicrobial properties of silver and copper nanoparticles were investigated using Escherichia coli (four strains), Bacillus subtilis and Staphylococcus aureus (Ruparelia et al., 2008). In literature, lot of study has been done to see the effect of AgNPs on bacteria (Lara et al., 2011; Illingworth et al., 2000; Lara et al., 2010; Petrus et al., 2011; Ansari et al., 2011; Prakash et al., 2011; Egorova, 2011). Silver and copper nanoparticles supported on various suitable materials, such as carbon, polyurethane foam and polymers have also been effectively used for bactericidal applications (Kumar et al., 2005; Jain and Pradeep, 2005).

Recently many reports clearly revealed the broad spectrum antibacterial activity of AgNPs against both Gram positive and Gram negative bacteria including multi resistant strains (Panacek et al., 2009). It is worth mentioning that AgNPs are performing multiple modes of inhibitory action to microorganisms rather than single specific action of antibiotics (Kim et al., 2008; Jo et al., 2009; Gogoi et al., 2006). Interestingly, AgNPs are effective against Candida spp., Dermatophytes and a few phytopathogenic fungi including Bipolaris sorokiniana and Magnapothe grisea [Panacek et al., 2006; Morones et al., 2005; Shrivastava et al., 2007]. On the other hand, many phytopathogenic fungi
are not explored although they are causing dreadful diseases on important crop plants and thereby reducing the yield of agricultural products.

Hence the present study was aimed to determine the antibacterial activities of biologically synthesized AgNPs against Gram positive and Gram negative bacteria and antifungal activities against phyto-pathogenic fungi.

### 2.10.3. Application of biogenic silver nanoparticles on denim fabrics

In view of the textile industry's innovative history nanotechnology has found its way into this sector quickly. The novel properties and low material consumption amount has attracted global interest across disciplines and industries. The textile sector is no exception (Rajendran *et al.*, 2010). Clothing and textile materials are good media for growth of microorganisms such as bacteria and fungi. According to recent reports, microorganisms could survive on fabric materials for more than 90 days in a hospital environment. Such a high survival rate of pathogens on medically used textiles may contribute to transmissions of diseases in hospitals (Abdel-mohsen *et al.*, 2012). These textile materials have generated a considerable interest in the medical field where these wide range of materials in the form of monofilament, multifilament, woven or nonwoven structures are being used as sutures, bandages, scaffolds, wound dressing, masks, surgical gowns and hospital linen, etc.

A lot of commercial medical textile products are available with antimicrobial property wherein the growth of micro-organisms is controlled by treatment with antimicrobial agents. Antimicrobial agents can also be integrated in textiles substrates to make these rot proof, mildew stain proof and to prevent perspiration odor resulting from microbial growth on textiles. The major classes of synthetic antimicrobial agents for textiles include triclosan, metal and their salts, organometallics, phenols, quaternary ammonium compounds and organosilicons, etc. (Purwar and Joshi, 2004). Antimicrobial textiles using chitosan nanoparticles (Ali *et al.*, 2011), ZnO nanoparticles (Rajendran *et al.*, 2010) AgNPs (Kulthong *et al.*, 2010) etc are reported in the literature.

The current study was done to see the effect of AgNPs on denim fabrics. The scope for denim wear is increasing tremendously every year and its worldwide market share has increased unpredictably in the last few decades. Consumers’ needs are fine tuned towards the latest developments and new styles; they are also aware of special
finishes and process treatments given to the garment to make them eco-friendly and user friendly (Sumithra and Raaja, 2012). The present study focuses on screening for the antimicrobial activity of AgNPs and providing the denim fabric with antimicrobial finish.

2.10.4. Application of biogenic silver nanoparticles in photocatalytic dye degradation

Textile dye effluents are dark in color having high BOD and COD content. Besides these, other chemicals like caustic soda, mordant, soap etc are also present in untreated dye effluent (Malviya et al., 2012). Dyes released from the textile processing and dye stuff manufacturing industries result in an increase in organic load of natural reservoirs. Generally, various dyes found in industrial effluents, ultimately, enter the aquatic ecosystem and can create various environmental hazards (Gandhi et al., 2010). The dyes are toxic and carcinogenic in nature and environmental contamination by these toxic chemicals is emerging as a serious global problem. Colored solution containing dyes from industrial effluents of textile, dyeing and printing industries may cause skin cancer due to photosensitization and photodynamic damage (Kansal et al., 2008). The chemical analysis of industrial waste water is essential to assess the degree of environmental pollution or toxicity caused by the industrial effluents (Sarwar et al., 2011). Currently textile effluents are treated by physicochemical methods that are often quite expensive; in addition, these methods do not generally degrade the pollutants thereby causing accumulation of dyes as sludge that creates a disposal problem (Manikandan et al., 2012).

Physical and chemical methods of dye removal are effective only if the effluent volume is small. This limits the use of physio-chemical methods, such as membrane filtration and cucurbituril, to small-scale in situ removal. A limiting factor of these methods is cost. This is true even in lab-scale studies, and methods, therefore, are unable to be used by large-scale industry. Biological activity, in liquid state fermentations, is incapable of removing dyes from effluent on a continuous basis. This is due to the time period of a few days required for decolourisation-fermentation processes. In order for this to be a viable option for industry, the dye-containing effluent must be held in large tanks (Robinson et al., 2001). Sunlight is a natural energy source that is abundant and can be exploited for degradation of dye effluent. Compared to other techniques, solar light was
found to be faster in decolorizing dye in the presence of metal catalyst (Kansal et al., 2008).

The acceleration of a chemical transformation in the presence of a catalyst with light is called photocatalysis. The catalyst may accelerate the photoreaction by interaction with the substrate in its ground or excited state and/or with a primary photoproduct, depending upon the mechanism of the photoreaction and itself remaining unaltered at the end of each catalytic cycle. The catalyst takes part in the redox reaction to form primary oxidizing species in the photocatalytic oxidation processes-hydroxyl radical (\(\cdot OH\)) and superoxide radical anions (\(O_2^{\cdot -}\)). These oxidative reactions would result in the degradation of the pollutants as shown in the following oxidation of the pollutants via successive attack by OH radicals

\[
R + \cdot OH \rightarrow R^{\cdot +} + H_2O
\]

or by direct reaction with holes (Haque et al., 2012)

\[
R + h^+ \rightarrow R^{\alpha +} \rightarrow \text{degradation products}
\]

*Phaseolus vulgaris* Linn. is a herbaceous annual plant grown worldwide. Brazil and India are the largest producers of dry beans. Seed germination is a critical stage that ensures reproduction and controls the dynamics of plant populations, thus it is a critical test of probable crop productivity (Dash, 2012). This water is discharged from industries directly into surrounding water bodies without any treatment and pollutes the water. This water is again used for irrigation affecting the seed germination and overall growth of plants (Sarwar et al., 2011).

In view of such perspectives, the present investigation was conducted to evaluate the impact of photo catalytically degraded commercial textile dye effluent using biogenic AgNPs.

### 2.10.5. Application of biogenic silver nanoparticles in wastewater treatment

An emerging technology for the abiotic degradation of recalcitrant compounds involves the usage of zerovalent metal and bimetallic systems. Zerovalent metals have been used for the reductive removal or transformation of organic compounds (Vyjayanthi, 2012). In the area of water purification, nanotechnology offers the possibility of an efficient removal of pollutants and germs. Today nanoparticles, nanomembrane and nanopowder used for detection and removal of chemical and
biological substances include metals (e.g. Cadmium, copper, lead, mercury, nickel, zinc), nutrients (e.g. Phosphate, ammonia, nitrate and nitrite), cyanide, organics, algae (e.g. cyanobacterial toxins) viruses, bacteria, parasites and antibiotics. It is suggested that these may be used in future at large scale water purification (Tiwari et al., 2008).

Many studies have recently focused on using natural polymers as support for catalyst preparation due to three important features: 1) formation of nanoparticles under mild and eco-friendly conditions 2) certain supports themselves act as a reductant for the conversion of metallic ions into their zerovalent forms 3) the resulting bio-inorganic catalyst can be easily separated from the reaction medium which promotes its reusability (Vyjayanthi, 2012). As per literature, studies have already been done to see the effect pollutant treatment by chitosan hollow fibres and beads (Guibal 2005, Liu et al., 2010), calcium alginate bead (Saha et al., 2010), Cobalt and Nickel nanoparticles inside poly (2-acrylamido-2-methyl-1-propansulfonic acid) (p(AMPS)) hydrogel (Sahiner et al., 2010) etc.

The focus in the current research is to incorporate biogenic AgNPs in calcium alginate beads for the reductive remediation of recalcitrant pollutants.

2.10.6. Phytotoxic effects of biogenic silver nanoparticles on plants

Although nanotechnology is quite a recent discipline, there have already high number of publications which discuss this topic. However, the safety of nanomaterials is of high priority. Whereas toxicity focuses on human beings and aims at protecting individuals, ecotoxicity looks at various trophic organism levels and intend to protect populations and ecosystems. Ecotoxicity includes natural uptake mechanisms and the influence of environmental factors on bioavailability (and thereby on toxicity) (Rana and Kalaichelvan, 2013). Nanomaterials end up in the environment, that is, in water, sediments, and soil (Oberdorster, 2004).

Phytotoxicity of nanoparticles has been demonstrated for Zn and ZnO as inhibition of seed germination and root growth after 2 hr exposure to nanoparticle suspensions in deionized water (Lin and Xing, 2007). Five types of nanoparticles (multiwalled carbon nanotubes, aluminum, alumina, zinc, and zinc oxide) and six plant species (radish, rape, ryegrass, lettuce, corn, and cucumber) were screened. Fifty percent inhibition of root growth (the most sensitive parameter) was observed for nano-Zn and
nano-ZnO at approximately 50mg/L for radish, and about 20 mg/ L for rape and ryegrass whereas other nanoparticle-plant combinations showed weaker inhibition. Results have shown that pure alumina particles significantly reduce the root elongation in all plant species, thus potentially slowing the plants’ growth.

Nanoparticles in plants enter cellular system via roots and stomata, effect transpiration, plant respiration, and photosynthesis, and interfere with translocation of food material. The degree of toxicity is linked to this surface and to the surface properties of these nanoparticles, rather than their mass. Hence, the most urgent needs for research related to environmental impact of nanoparticles are to establish the degree of environmental mobility and bioavailability. These parameters will decide whether nanoparticles can be taken up and cause harm to various organisms especially plants (Rana and Kalaichelvan, 2013).

*Trigonella foenumgraecum* Linn. (Fenugreek) is a legume, originally from southeastern Europe and western Asia, but grown now mainly in India and also in certain parts of Asia, northern Africa, Europe and the United States (Altuntas *et al.*, 2005). It is a winter season crop and grows well in low temperature. Fenugreek seeds are traditionally used for the treatment of many diseases. Studies show that the seeds have antioxidant properties. Many medicinal properties are attributed to fenugreek seeds and leaves (Srinivasan, 2006). Fenugreek is known to have several pharmacological attributes such as hypoglycaemic (Sharma and Raghuram, 1990; Zia *et al.*, 2001), hypercholesterolaemic (Stark and Madar, 1993; Srinivasan, 2006), gastroprotective (Suja Pandian *et al.*, 2002), chemo-preventive (Amin *et al.*, 2005), antioxidant (Hettiarachchy *et al.*, 1996; Kaviarasan *et al.*, 2007), laxative (Riad and El- Baradie, 1959) and appetite stimulation (Petit *et al.*, 1993). The plant is known to contain alkaloids (Jain and Madhu, 1988), flavonoids (Kamal and Yadav, 1991), salicylate (Swain *et al.*, 1985), and nicotinic acid (Rajalaksmi *et al.*, 1964).

There are reports of metal stress on this plant like lead and cadmium (Suganthi *et al.*, 2013; Swamy *et al.*, 2011). Girilal (2013) showed that biogenic AgNPs showed lesser toxicity when compared to chemically synthesized AgNPs in *Solanum lycopersicum*. However, there are no reports on phytotoxic effect of bioaccumulation of biogenic
AgNPs. Hence the current research was carried out to see the phytotoxic effects of AgNPs on *Trigonella foenumgraecum* Linn. using biogenic AgNPs.