Review Article

Silver Nanoparticles - A Review with Focus on Green Synthesis

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ABSTRACT

The present review focuses on the green synthesis of silver nanoparticles by using an endophytic fungus, the properties of silver nanoparticles and the general techniques by which nanoparticles can well characterized. Silver has been used since ancient time due to its potent antimicrobial effect and now it is recognized as a non toxic and safe for human beings. Silver nanoparticles have attracted keen interest due to its specific size ranges 1-100 nm and its unique physical, chemical and biological properties. There are several methods reported for the synthesis of silver nanoparticles including physical, chemical and biological methods in which biological method are cheap, reliable, safe and non toxic over physical and chemical methods. Furthermore green synthesis technique is a promising approach for synthesis of silver nanoparticles showing antimicrobial effect as its not uses any toxic chemicals and specific higher temperature and pressure. But use of endophytic Fungi provides shield and survival condition to their host plants by secreting large amount of protein which can in a straight way convert to higher output of nanoparticles as well as can avoid the quick lessening of plant sources. These are characterized for physical and chemical properties of silver nanoparticles such as optical, magnetic and catalytic properties etc. by which nanoparticles shows influence in their reactivity as compared to macrostructures.

Keywords: Silver, green synthesis, nanoparticles, endophyte

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INTRODUCTION:

Nanoparticles are the clusters of atoms in the size range of 1–100 nm. In this size range, materials often develop useful attributes that are distinct from the properties of the bulk material. Metal particles in the nanometer size exhibit unique physical properties that are different from both the ion and the bulk material. Their uniqueness arises specifically from higher surface to volume ratio which results in increased catalytic activity due to morphologies with highly active facets; hence, the nanosize materials are more advantageous than their bulk materials [1]. As the size of matter is reduced to tens of nanometres or less, quantum effects can begin to play a role, and these can significantly change a material's optical, magnetic or electrical properties. In some cases, size-dependent properties have been exploited for centuries. For example, gold and silver nanoparticles (particles of diameter less than 100 nm; have been used as colored pigments in stained glass and ceramics since the 10th century AD (Erhardt 2003) The challenge for the ancient chemists was to make all nanoparticles the same size (and hence the same color), and the production of single-size nanoparticle is still a challenge today [2]. At the larger end of our size range, other effects such as surface tension or 'stickiness' are important, which also affect physical and chemical properties. For liquid or gaseous environments Brownian motion, which describes the random movement of larger particles or molecules owing to their bombardment by smaller molecules and atoms, is also important. This effect makes control of individual atoms or molecules in these environments extremely difficult. The concept of nanotechnology though considered to be a modern science has its history dating to as back as the 9th

century. Nanoparticles (NPs) of gold and silver were used by the artisans of Mesopotamia to generate a glittering effect to pots. The first scientific description of the properties of nanoparticles was provided in 1857 by Michael Faraday in his famous paper "Experimental relations of gold (and other metals) to light". In 1959, Richard Feynman gave a talk describing molecular machines built with atomic precision. This was considered the first talk on nanotechnology. This was entitled "There's plenty of space at the bottom." [3]

In last decay, application of nano material has been extensively increased in high demand leads to the bulk production of the nanomaterial. Classically, the nanoparticles are produced by physical method needs more energy and chemical methods are toxic but biological methods are clean, safe and cost effective. Biological methods of nanoparticles synthesis using microorganism, enzyme and plant or plant extract have been suggested as possible ecofriendly alternative to chemical and physical methods. It can also be suitable for large-scale synthesis of nanoparticles. There are several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra- or extracellularly, and thus to be potentially utilized as eco-friendly nanofactories. [4]

A number of bacteria like Bacillus subtilis, Pseudomonas stutzeri, Thermonospora sp., Shewanella algae Lactobacillus strains, etc. have been studied for the synthesis of metallic nanoparticles. Yeast has also been explored for the biosynthesis of nanoparticles including Candida glabrata, Schizosaccaharomyces pombe, MKY3 etc. While, a number of plants like Medicago sativa, Pelargonium graveolans, Azadirachta indica, Triticum, Cinnamomum camphora, Capsicum annum have been used for the fabrication of metal nanoparticles [5]. Nanoparticles produced by a bio-enzymatic process are far superior, in several ways, to those particles produced by chemical methods despite the fact that the latter methods are able to produce large quantities of nanoparticles with a defined size and shape in a relatively short time. With an enzymatic process, the use of expensive chemicals is eliminated, and the more acceptable "green" route is not as energy intensive as the chemical method and is also environment friendly. Nanoparticles are biosynthesized when the microorganisms grab target ions from their environment and then turn the metal ions into the element metal through enzymes generated by the cell activities. It can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are formed [6]. The synthesis process is normally divided into two broad groups: the 'top-down' and the 'bottom-up' approaches. The top-down approach involves reducing the size of bulk materials by employing mechanical, chemical or other forms of energy. The breakdown of large structures can be achieved by use of ultra fine grinders, lasers and vaporization followed by cooling. Powerful techniques of lithography and etching start with large uniform pieces of material and generate the required nanostructures from them. The bottom-up approach on the other hand involves synthesizing nanomaterials from atomic or molecular species via self - assembly or chemical reactions allowing the precursor particles to increase in size. It is very powerful in creating identical structures with atomic precision [7]. The intend of the present review was focus on the green synthesis of silver nanoparticles by using an endophytic fungi, its properties and general characterization techniques.

SILVER NANOPARTICLES:

The medical properties of silver have been known for over 2,000 years. Since the nineteenth century, silver-based compounds have been used in many antimicrobial applications. Nanoparticles have been known to be used for numerous physical, biological, and pharmaceutical applications. Silver nanoparticles are being used as antimicrobial agents. Silver nanoparticles are nanoparticles of silver which are in the range of 1 and 100 nm in size. Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures [8]. Silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties

compared to their macro-scaled counterparts. Ag-NPs have distinctive physico-chemical properties, including a high electrical and thermal conductivity, surface-enhanced Raman scattering, chemical stability, catalytic activity and non linear optical behaviour. These properties make them of potential value in inks, microelectronics, and medical imaging. Besides, Ag-NPs exhibit broad spectrum bactericidal and fungicidal activity that has made them extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food and textiles, increasing their market value [9]. Silver is one of the basic element that makes up our planet. It is a rare, but naturally occurring element, slightly harder than gold and very ductile and malleable. AgNPs of many different shapes (spherical, rod-shaped, truncated, triangular nanoplates) were developed by various synthetic routes. Truncated triangular silver nanoplates were found to show the strongest anti-bacterial activity. The AgNPs have excellent antimicrobial property compared to other salts due to their extremely large surface area, which provides better contact with microorganisms [10].

PROPERTIES OF SILVER NANO-PARTICLES:

Many unusual properties of nanomaterials arise due to spatial confinement of electrons, photons, and electric fields around the particles as well as due to their large surface to volume ratio. The physical and chemical properties of nanoparticles depend on the type of motion its electrons can execute, which is dictated by the degree of their spatial confinement. When compared to macrostructures, larger proportions of constituent atoms or molecules lie at the surface of nanomaterials, leading to large surface to volume ratio, which can influence their reactivity, hardness, as well as magnetic, catalytic, and opto-electronic properties. [11]

Some of the unique properties exhibited by such NPs are as follows:

OPTICAL PROPERTIES: Metal nanoparticles show different optical properties corresponding to their bulk form [12]. These properties are dependent on composition, size, shape and surrounding medium of the nanoparticles [13]. Depending on the size and shape of the nanoparticles, the optical properties vary from visible region to NIR region..The optical properties of colloidal nanoparticles in the UV-Vis-NIR spectral range are mainly determined by the so-called localized surface plasmon resonance (LSPRs) [14]

SURFACE PLASMON RESONANCE: Electromagnetic (EM) radiations cause oscillations of conduction electrons leading to a phenomenon called Surface Plasmon resonance (SPR). Free electrons on the metal nanoparticles have Plasmon resonances which are a result of EM radiations. When the frequency of the incident light photons matches with that of the free electrons oscillating, a resonance condition occurs. Unique intense colours are exhibited by gold and silver nanoparticles as they possess free electrons and the SPR bands can be observed by using spectrophotometry. The shape and size of the nanoparticles greatly affect the colour exhibited which is also reflected in the spectra. Slight modifications in the geometry of the nanoparticles can cause significant changes in the colour. Thus, the SPR exhibited by anisotropic nanoparticles is significantly distinguishable from that of isotropic (spherical) nanoparticles. While nanospheres typically have Plasmon resonance oscillations at one specific wavelength, anisotropic particles have both transverse and longitudinal oscillations that occur at different wavelengths of light. Thus, in the spectra of anisotropic nanoparticles, two separate SPR bands will be detected whereas only a single peak is displayed in case of spherical nanoparticles . While the longitudinal Plasmon band is related to the light absorption & scattering along the long axis of the particle, the transverse Plasmon band is contributed by the light absorption & scattering along the short axis of the particle. [15]

CATALYTIC ACTIVITY: Large surface areas of the nanomaterials make them good for adsorption at specific sites. Anisotropic nanostructures exhibit different crystal surfaces, and different fractions of atoms are located at different edges and corners. Thus, the catalytic efficiency is expected to

be different in catalyzing the same reaction [16]. Different morphologies of platinum NPs had enhanced and selective catalytic activities when compared to their spherical counterparts [17].

MAGNETIC PROPERTIES:

Superparamagnetism is a form of magnetism that is a special characteristic of small ferromagnetic or ferromagnetic nanoparticles. In such superparamagnetic nanoparticles, magnetization can randomly change direction under the influence of temperature. Superparamagnetism occurs when a material is composed of very small particles with a size range of 1- 10nm. In the presence of an external magnetic field, the material behaves in a manner similar to paramagnetism with an exception that the magnetic moment of the entire material tends to align with the external magnetic field [3].

MELTING TEMPERATURE:

The bulk melting temperature is independent on its size. However, melting temperature for NP depends on its dimension and their melting temperature is lower than the corresponding bulk materials. It is known that the melting temperature depression result from the high surface-to-volume ratio and the surface substantially affects the interior "bulk" properties of these materials. Since the melting temperature depression results from the large surface-to-volume ratio, the surface areas of nanoparticles in different shape will be different even in the identical volume, and the area difference is large especially in small particle size. The melting temperature depression of nanoparticles is apparent only when the particle size is smaller than 100 nm. If the particle size is larger than 100 nm, the melting temperature of the particles approximately equals to the corresponding bulk materials, and in other words, the melting temperature of nanoparticles is independent of the particle size. It is found that the particle shape can affect the melting temperature of nanoparticles, and this effect on the melting temperature become larger with decreasing of the particle size [18].

SYNTHESIS OF SILVER NANOPARTICLES:

PHYSICAL APPROACHES:

Most important physical approaches include evaporation-condensation and laser ablation. Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide have previously been synthesized using the evaporation-condensation method. The absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution are the advantages of physical approaches in comparison with chemical processes. Physical synthesis of silver nanoparticles using a tube furnace at atmospheric pressure has some disadvantages, for example, tube furnace occupies a large space, consumes a great amount of energy while raising the environmental temperature around the source material, and requires a great deal of time to achieve thermal stability. Moreover, a typical tube furnace requires power consumption of more than several kilowatts and a preheating time of several tens of minutes to reach a stable operating temperature [19].

CHEMICAL APPROACHES:

Chemical methods have been mostly used for production of Ag-NPs. Chemical methods provide an easy way to synthesize Ag-NPs in solution. Monodisperse samples of silver nanocubes were synthesized in large quantities by reducing silver nitrate with ethylene glycol in the presence of polyvinylpyrrolidone (PVP), the so-called polyol process. In this case, ethylene glycol served as both reductant and solvent. It showed that the presence of PVP and its molar ratio relative to silver nitrate both played important roles in determining the geometric shape and size of the product. It suggested that it is possible to tune the size of silver nanocubes by controlling the experimental conditions. Spherical Ag-NPs with a controllable size and high monodispersity were synthesized by using the polyol process and a modified precursor injection technique. In the precursor injection method, the injection rate and reaction temperature were important factors for producing uniform-sized Ag-NPs with a reduced size. Ag-NPs with a size of 17±2 nm were obtained at an injection rate

of 2.5 ml s−1 and a reaction temperature of 100 °C. The injection of the precursor solution into a hot solution is an effective means to induce rapid nucleation in a short period of time, ensuring the fabrication of Ag-NPs with a smaller size and a narrower size distribution. Nearly monodisperse Ag-NPs have been prepared in a simple oleylamine-liquid paraffin system. It was shown that the formation process of Ag-NPs could be divided in the three stages: growth, incubation and Ostwald ripening stages. In this method, only three chemicals, including silver nitrate, oleylamine and liquid paraffin, are employed throughout the whole process. Generally, the chemical synthesis process of the Ag-NPs in solution usually employs the following three main components: (i) metal precursors, (ii) reducing agents and (iii) stabilizing/ capping agents. The formation of colloidal solutions from the reduction of silver salts involves two stages of nucleation and subsequent growth. It is also revealed that the size and the shape of synthesized Ag-NPs are strongly dependent on these stages. Furthermore, for the synthesis of monodispered Ag-NPs with uniform size distribution, all nuclei are required to form at the same time. In this case, all the nuclei are likely to have the same or similar size, and then they will have the same subsequent growth. The initial nucleation and the subsequent growth of initial nuclei can be controlled by adjusting the reaction parameters such as reaction temperature, pH, precursors, reduction agents (i.e. NaBH4, ethylene glycol, glucose) and stabilizing agents (i.e. PVA, PVP, sodium oleate [20].

GREEN SYNTHESIS OF SILVER NANOPARTICLES:

In recent years, the development of efficient green chemistry methods employing natural reducing, capping, stabilizing agents to prepare silver nanoparticles with desired morphology and size have become a major focus of researchers. Biological methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances [21-22].

SYNTHESIS OF SILVER NANOPARTICLES BY PLANTS:

The major advantage of using plant extracts for silver nanoparticle synthesis 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 in the synthesis. The main mechanism considered for the process is plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions. Studies have revealed that xerophytes contain emodin, an anthraquinone that undergoes tautomerization, leading to the formation of the silver nanoparticles. In the case of mesophytes, it was found that they contain three types of benzoquinones: cyperoquinone, dietchequinone, and remirin. It was suggested that the phytochemicals are involved directly in the reduction of the ions and formation of silver nanoparticles [23].

SYNTHESIS OF SILVER NANOPARTICLES BY BACTERIA:

Bacteria are known to produce inorganic materials either intra- or extracellularly. This makes them potential biofactories for the synthesis of nanoparticles like gold and silver. Silver is well known for its biocidal properties; however, some bacteria are known to be silver resistant and can accumulate silver on the cell wall to as much as 25% of their dry weight biomass, thus suggesting their use in industrial recovery of silver from ore materials. Therefore, the use of prokaryotic bacteria as nanofactories was first studied [24]. The first evidence of bacteria synthesizing silver nanoparticles was established using the *Pseudomonas stutzeri* AG259 strain that was isolated from silver mine [25]. There are some microorganisms that can survive metal ion concentrations and can also grow under those conditions, and this phenomenon is due to their resistance to that metal. The mechanisms involved in the resistance are efflux systems, alteration of solubility and toxicity via reduction or

oxidation, biosorption, bioaccumulation, extracellular complex formation or precipitation of metals, and lack of specific metal transport systems [26].

SYNTHESIS OF SILVER NANOPARTICLES BY FUNGI:

When in comparison with bacteria, fungi can produce larger amounts of nanoparticles because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles [27]. The mechanism of silver nanoparticle production by fungi is said to follow the following steps: trapping of Ag+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system [28]. The extracellular enzymes like naphthoquinones and anthraquinones are said to facilitate the reduction. Considering the example of F. oxysporum, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation [29].

SYNTHESIS OF SILVER NANOPARTICLES USING ENDOPHYTIC FUNGI:

Endophytic fungi are microorganisms which spend the whole or part of their life-cycle residing symbiotically within the healthy tissues of host plants, inter-and/or intra-cellularly and produce bioactive natural products or drugs and derivatives; meanwhile causing no damage or disease to their hosts [30]. Taken literally, the word endophyte means “in the plant” (endon Gr. = within, phyton = plant). The usage of this term is as broad as its literal definition and spectrum of potential hosts and inhabitants, e.g. bacteria (Kobayashi and Palumbo 2000), fungi (Stone et al. 2000), plants (Marler et al. 1999) and insects in plants (Feller 1995), but also for algae within algae (Peters 1991). Endophytic fungi are an ecological, polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamorphic fungi. Approximately, there are near to 300,000 plant species on earth and each individual plant is the host to one or more endophytes, and many of them may colonize certain hosts. It has been estimated that there may be as many as one million different endophytic fungal taxa, thus endophytes may be hyperdiverse. The presence of biodiverse endophytes in huge number plays an important role on ecosystems with greatest biodiversity. Endophytes provide a broad variety of bioactive secondary metabolites with unique structure, including alkaloids, benzopyranones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones, xanthones, and others [31]. The endophytes may provide protection and survival condition to their host plant by producing a plethora of substances which once isolated and characterized, may also have potential for use in industry, agriculture and medicine [32]. Some species of endophytic fungi have been identified as sources of anticancer, antidiabetic, insecticidal and immuno-suppressive Some studies show that the endophytes are not host specific a single endophyte can survive a wide range of host. A large number of fungi isolated from the different parts of the same plants which differ in their ability to utilize different substances [33]. Therefore, a number of fungi can be isolated from different plant belonging to different genera and grow under different climatic conditions. The host and endophytes relationship varies from host to host and endophytes. Some studies show that host plant and endophyte relationship also able to maintain the pathogen host antagonism [34].

Table 1: Examples of biosynthesized nonmaterial’s using Fungi

Aspergillus niger	Ag 20	Gade et al.
Aspergillus oryzae	Ag 5-50	Binupriya et al.
Fusarium oxysporum	Ag 1-5	Duran et al.
Fusarium solani	Ag 5-35	Ingle et al.
Pleurotussajor-caju	Ag 5-50	Nithyaand Ragunathan
Trichoderma viride	Ag 10-40	Thakkar et al.
Klebsiella pneumonia	Se 100-400	Fesharaki et al.

NEED FOR GREEN SYNTHESIS:

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The need for biosynthesis of nanoparticles is as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route. So, in the search of cheaper pathways for nanoparticles synthesis, scientist used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties they are usually responsible for the reduction of metal compounds into their respective nanoparticles. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [35].

MECHANISM OF MYCOSYNTHESIS OF NANOPARTICLES

Fungi can produce nanoparticles both extracellularly as well as intracellularly however the exact mechanism is not understood completely. Putative mechanisms during intracellular synthesis include heavy metal binding to fungal cell wall by proteins or enzymes present on it via electrostatic interactions. Furthermore, the metal ions are reduced by enzymes present in cell wall. This leads to aggregation of metal ions and formation of nanoparticles. Extracellular synthesis assumed interaction of metal ions and release of enzyme mainly reductase with subsequent formation of nanoparticles in solution [36]. Extracellular synthesis of nanoparticles has advantages as it does not require lysis of fungal cell, downstream processing for recovery and purification of nanoparticles [37]. Whereas, in case of intracellular synthesis recovery and purification of nanoparticles from fungi biomass is tedious task and hence analytical equipments and long processing techniques are required. There was a specific role of enzyme alpha NADPH dependent nitrate reductase in AgNPs synthesis [38]. The Ag+ ions were reduced by nitrate reductase leads to formation of silver nanoparticles The reduction of silver ions (Ag+) in aqueous solution generally yields colloidal silver with particles diameters of several nanometers. This crystallisation process initially involves the reduction of (Ag+) ions to silver atoms (Ag 0) which is followed by the growth of crystal into oligomeric clusters. These clusters eventually lead to the formation of colloidal Ag particles. When the colloidal particles are much smaller than the wavelength of visible light, the solution absorbs at a shorter wavelength in the range of 380-400 nm. As the particles size increases this band shifts to longer wavelength in the absorbtion spectrum. This band is the result of collective oscillation of the electron gas in the particles, phenomenon known as surface Plasmon resonance. Efficacy of silver nanoparticles Size dependent and attempts have been made to control the particles size and modulating their shape. The green synthesis of Ag NPs involves three main criteria which must be evaluated based on the green chemistry perspectives include (1) choice of solvent medium (2) variety of environmentally benign reducing agent and (3) range of nontoxic substances for the Ag Nps stability. Based on this approach, we can proceed for green – chemistry type Ag NP synthesis process. Nanoparticles can be synthesized using various approaches including chemical, physical, and biological. [38]

GENERAL TECHNIQUES FOR CHARACTERIZATION OF SILVER NANOPARTICLES:

The metal NPs are characterized by different techniques such as:

1. UV-Vis-NIR Spectroscopy
2. Transmission Electron Microscopy
3. Scanning Electron Microscopy
4. X-Ray Diffraction
5. Fourier transform infra red spectroscopy
6. X-Ray photoemission spectroscopy etc.

Some of the techniques are discussed in the following sections:

UV-VIS-NIR SPECTROSCOPY: It can be used as a means to describe noble metal nanoparticles. In general, it is a technique

used to compute the light that is absorbed and scattered by a sample (a quantity known as the extinction, which is defined as the sum of absorbed and scattered light).Nanoparticles have optical properties that are responsive to size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, which makes UV/Vis/NIR spectroscopy a important tool for identifying, characterizing, and studying these materials. In its simplest form, a sample is placed between a light source and a photo-detector, and the intensity of a beam of light is calculated before and after passing through the sample. Qualitative information about the nanoparticle solution can be obtained by this technique. The absorbance is obtained by employing Beer-Lambert Law. The absorbance value (A) depends on nanoparticle concentration (c), path length (l) of measuring cell and extinction coefficient of nanopaticles ( )

A= Cl

The transmittance of a sample (*T*) is defined as the fraction of photons that pass through the sample over the incident number of photons, i.e., $T = I/I0$.In a typical UV/vis/IR spectroscopy measurement, those photons are measured that are not absorbed or scattered by the sample. The absorbance (*A*) of the sample is related to the transmittance by $A = -\log10 (T)$. As a general rule, smaller particles will have a higher percentage of their extinction due to absorption. UV-Visible spectroscopy can be used as a simple and reliable method for monitoring the stability of nanoparticle solutions also. As the particles destabilize, the original extinction peak will decrease in intensity (due to the depletion of stable nanoparticles), and often the peak will broaden or a secondary peak will form at longer wavelengths (due to the formation of aggregates [39].

TRANSMISSION ELECTRON MICROSCOPY (TEM): It is a vital characterization tool for directly imaging nanomaterials to obtain quantitative measures of particle size, size distribution, and morphology. It operates on the same fundamental philosophy as the light microscope but uses electrons instead of light. The resolution in case of light microscope is limited by the wavelength of light. But Transmission electron microscopes use electrons as "light source" and their much lower wavelength makes it possible to get a resolution a thousand times better than with a light microscope. A "light source" at the top of the microscope emits the electrons that pass through vacuum in the column of the microscope. Instead of glass lenses focusing the light in the light microscope, the TEM uses electromagnetic lenses to focus the electrons into a very thin beam. The electron beam then travels through the sample of study. Depending on the density of the substance present, some of the electrons are scattered and fade away from the beam. At the bottom of the microscope the unscattered electrons hit a fluorescent screen, which gives rise to a "shadow image" of the specimen with its different parts displayed in varied darkness according to their density. The image can be studied directly by the operator or photographed with a camera. Successful imaging of nanoparticles using TEM depends on the contrast of the sample relative to the background. Samples are arranged for imaging by drying nanoparticles on a copper grid that is layered with a thin layer of carbon. Materials with electron densities that are appreciably higher than amorphous carbon are easily imaged. These resources include most metals (e.g., silver, gold, copper, aluminum), most oxides (e.g., silica, aluminum oxide, titanium oxide), and other particles such as polymer nanoparticles, carbon nanotubes, quantum dots, and magnetic nanoparticles [40].

SELECTED AREA ENERGY DIFFRACTION (SAED): It is a crystallographic experimental technique that can be performed inside a TEM. An aperture in the image plane is used to select the diffracted region of the specimen, giving site-selective diffraction analysis. SAD patterns are a projection of the reciprocal lattice, with lattice reflections showing as sharp diffraction spots. By tilting a crystalline sample to low-index zone axis, SAD patterns can be used to identify crystal structures and measure lattice parameters. SAD of nanocrystals gives ring patterns analogous to those from X-ray powder diffraction, and can be used to

identify texture and discriminate nanocrystalline from amorphous phases. [52]

ENERGY DISPERSIVE X-RAY ANALYSIS (EDAX): It is a compositional analysis technique. The high energy electron beam can eject electrons from inner-shell atomic orbitals. The resulting vacancies are filled by electrons from higher energy shells. Electron energies lost during these transitions are emitted as X-rays. The transitions, and hence X-rays, have energies that are characteristic of the atomic species. Therefore, measuring the X-ray spectrum allows identification of the sample composition, and is typically sensitive down to levels of about 0.5 to 1 atomic percent. [52]

HIGH RESOLUTION TEM (HRTEM): In general, it refers to imaging in which lattice fringes (i.e. crystallographic planes) are observed or atomic resolution is achieved. HRTEM images are formed from a number of diffracted beams. This multi-beam approach is known as phase-contrast imaging, and is necessary to construct an image of the crystal lattice. HRTEM provides access to much information about the sample, such as analyzing crystalline defects and interfaces at the atomic scale, and observing and verifying devices, multilayer, nanocrystals and nanostructures. [52]

SCANNING ELECTRON MICROSCOPY (SEM): Unlike in a TEM where a stationary, parallel electron beam is used to form images, the SEM, is a mapping device. In a SEM instrument, a fine electron probe, formed by using a strong objective lens to de-magnify a small electron source, is scanned over a specimen in a two-dimensional raster. Signals generated from the specimen are detected, amplified, and used to modulate the brightness of a second electron beam that is scanned synchronously with the SEM electron probe across a cathode-ray-tube (CRT) display. Therefore, a specimen image is mapped onto the CRT display for observation. Since SEM is a serial recording instead of a parallel recording system, the whole process of generating a SEM image could be slower than that in the TEM.

X-RAY DIFFRACTION (XRD): It is the main method for crystallographic characterization for bulk, nano and thin film materials [41]. When X-rays interact with a crystalline substance (Phase), one gets a diffraction pattern. Every crystalline substance gives a pattern; the same substance always gives the same pattern and in a mixture of substances each produces its pattern independently of the others. Thus the XRD pattern of a pure substance is like a fingerprint of the substance. The powder diffraction method is therefore ideally suited for characterization and identification of polycrystalline phases. XRD is non contact and non-destructive, which makes it ideal for in situ studies. Nanomaterials have a characteristic microstructure length comparable with the critical length scales of physical phenomena, giving them unique mechanical, optical and electronic properties. X-ray diffractograms of nanomaterials provide a wealth of information - from phase composition to crystallite size, from lattice strain to crystallographic orientation. A typical powder XRD instrumentation consist of four main components such as X-ray source, specimen stage, receiving optics and X-ray detector. The source and detector with its associated optics lie on the circumference of focusing circle and the sample stage at the center of the circle. Bragg's law is the basis of XRD analysis. With this law it is possible to make accurate quantifications of experimental results in the determination of crystal structures. The angle between the plane of the specimen and the X-ray source is θ, known as Bragg's angle and the angle between the projection of X- ray and the detector is 2θ. . For the XRD analysis, fine powder samples were mounted on the sample holder and the powder was assumed to consist of randomly oriented crystallites. When a beam of X- ray is incident on the sample, X-rays are scattered by each atom in the sample. If the scattered beams are in phase, these interfere constructively and one gets the intensity maximum at that particular angle. The atomic planes from where the X-rays are scattered are referred to as 'reflecting planes'. There are some important differences between the diffraction patterns of nano and bulk materials. Nano materials have small particle size and this causes the

lines in their diffraction peak to broaden. The broadening of the peak is due to a small number of crystal planes. This broadening in turn causes a loss of intensity in the signal of their diffraction patterns. Bulk materials, on the contrary, have sharp, narrow and high-intensity peaks. Thus, XRD is an efficient analytical and rapid technique used to identify and characterize nano materials [42].

FOURIER TRANSFORM INFRARED SPECTROSCOY (FTIR):

The nanoparticles were subjected to FTIR analysis for analyzing the capping ligand of nanoparticles which act as a stabilizing agent. Synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal demonstrating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the natural molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the exterior of metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids [43].

X-RAY PHOTO EMISSION SPECTROSCOPY (XPS):

To investigate samples with higher precision and in some sense more locally XPS have to be used. This technique is classified as a surface sensitive because a typical analysis depth is limited to the thickness of ~7 nm. Because of such attenuation depth it seems to be perfect for performing investigations on clusters and nanoparticles. The general scan spectrum shows the presence of strong Cls, Ols, Nls, and Ag3d core levels. The Ag3d core level spectrum is resolved into two spin-orbit components, which occurred at binding energy of 368.0 and 375.0ev One can see that the most intense lines communicate to the core levels of carbon, silver and oxygen correspondingly like in case of EDX. This technique could also be used to obtain quantitative information about sample composition .The biggest advantage of the XPS is related with its possibility of detecting of chemical bonds which are in the sample [44-45].

ACTION OF SILVER NANOPARTICLES ON MICROBES(*ANTIMICROBIAL ACTIVITY* **):**

The exact mechanism which silver nanoparticles employ to cause antimicrobial effect is not clearly known and is a debated topic. There are however various theories on the action of silver nanoparticles on microbes to cause the microbicidal effect. The antimicrobial property of silver is related to the amount of silver and the rate of silver released. Silver in its metallic state is inert but it reacts with the moisture in the skin and the fluid of the wound and gets ionized. The ionized silver is highly reactive, as it binds to tissue proteins and brings structural changes in the bacterial cell wall and nuclear membrane leading to cell distortion and death .There is formation of 'pits' on the cell surface, and there is accumulation of the nanoparticles on the cell surface. The formation of free radicals by the silver nanoparticles may be considered to be another mechanism by which the cells die. There have been electron spin resonance spectroscopy studies that suggested that there is formation of free radicals by the silver nanoparticles when in contact with the bacteria, and these free radicals have the ability to damage the cell membrane and make it porous which can ultimately lead to cell death.

It has also been proposed that there can be release of silver ions by the nanoparticles, and these ions can interact with the thiol groups of many vital enzymes and inactivate them. The bacterial cells in

contact with silver take in silver ions, which inhibit several functions in the cell and damage the cells. Then, there is the generation of reactive oxygen species, which are produced possibly through the inhibition of a respiratory enzyme by silver ions and attack the cell itself. Silver is a soft acid, and there is a natural tendency of an acid to react with a base, in this case, a soft acid to react with a soft base. The cells are majorly made up of sulfur and phosphorus which are soft bases. The action of these nanoparticles on the cell can cause the reaction to take place and subsequently lead to cell death. Another fact is that the DNA has sulfur and phosphorus as its major components; the nanoparticles can act on these soft bases and destroy the DNA which would definitely lead to cell death. The interaction of the silver nanoparticles with the sulfur and phosphorus of the DNA can lead to problems in the DNA replication of the bacteria and thus terminate the microbes. It has also been found that the nanoparticles can modulate the signal transduction in bacteria. It is a well established fact that phosphorylation of protein substrates in bacteria influences bacterial signal transduction. Dephosphorylation is noted only in the tyrosine residues of gram-negative bacteria. The phosphor tyrosine profile of bacterial peptides is altered by the nanoparticles. It was found that the nanoparticles dephosphorylate the peptide substrates on tyrosine residues, which leads to signal transduction inhibition and thus the stoppage of growth. It is however necessary to understand that further research is required on the topic to thoroughly establish the claims. [46]

ANTIVIRAL EFFECTS:

In recent years, there was an increase in reported numbers of emerging and re-emerging infectious diseases caused by viruses such as SARS-Cov, influenza A/H5N1, influenza A/H1N1, Dengue virus, HIV, HBV, and new encephalitis viruses etc. These viral infections are likely to break out into highly infectious diseases endangering public health. Ag-NPs have shown effective activities against microorganisms including bacteria and fungi as mentioned above. However, the antiviral activities of Ag-NPs are still open questions to researchers. Very few papers have been found that investigate the effects of Ag-NPs against viruses. As the first report, Elechiguerra *et al* have investigated the interaction between Ag-NPs and HIV-1. It was reported that Ag-NPs undergo a size-dependent interaction, with NPs exclusively in the range of 1–10 nm attached to the virus. It was also suggested that Ag-NPs interact with the HIV-1 virus via preferential binding to the exposed sulfur-bearing residues of the gp120 glycoprotein knobs, resulting in the inhibition of the virus from binding to host cells. This mechanism was then demonstrated by Lara *et al*. In this article, it was reported that Ag-NPs exert anti-HIV activity at an early stage of viral replication, most likely as a virucidal agent or as an inhibitor of viral entry. The Ag-NPs bind to gp120 in a manner that prevents CD4-dependent virion binding, fusion, and infectivity, acting as an effective virucidal agent against cell-free virus (laboratory strains, clinical isolates, T and M tropic strains, and resistant strains) and cell-associated virus. Besides, Ag-NPs inhibit post-entry stages of the HIV-1 life cycle. [47-48]

ANTIMICROBIAL GEL FORMULATION FOR TOPICAL USE

In addition, Ag-NPs were also used in therapeutics, especially for treating burn wounds. In order to develop this test, a gel formulation containing Ag-NPs (S-gel) was developed. The antibacterial spectrum of S-gel was found to be comparable to that of a commercial formulation of silver sulfadiazine, albeit at a 30-fold less silver concentration. As part of toxicity studies, localization of Ag-NPs in Hep G2 cell line, cell viability, biochemical effects and apoptotic/necrotic potential were assessed. It was found that Ag-NPs get localized in the mitochondria and have an IC50 value of 251μg ml−1. Further, it was obvious that Ag-NPs induced apoptosis at concentrations up to 250μg ml−1, which could favor scar less wound healing. Acute dermal toxicity studies on Ag-NPs gel formulation (S-gel) in Sprague-Dawley rats showed complete safety for topical application. These results clearly indicated that Ag-NPs could provide a safer alternative to conventional

antimicrobial agents in the form of a topical antimicrobial formulation [49]

REGULATION OF NANOPARTICLES:

Products made with silver nanoparticles have been approved by a range of accredited bodies such as US Food and Drug Administration (FDA), US Environmental Protection Agency (EPA), and Research Institute for Chemical Industry and FITI Testing. Silver nanoparticles can be exploited in medicine and pharmacy for dental materials, burn treatments, coating stainless steel materials, textile fabrics, and sunscreen lotions . Silver nanoparticles are widely used in surface enhanced Raman scattering (SERS) spectroscopy (surface-sensitive technique which is known for its high sensitivity due to its surface plasmon effects lacks behind at uniform substrate formation) because of high local field enhancement factor. Silver Nanoparticles demonstrated that SERS and silver nanorods quickly revealed the viral structures. It can also differentiate between respiratory viruses, virus strains, and viruses containing gene deletions without manipulating the virus. Silver nanoparticles can also be used for modification of polymer based diffraction gratings which further can be used in grating light reflection spectroscopy for sensing [50].

TOXICITY OF SILVER NANOPARTICLES PRODUCED BY CHEMICAL AND PHYSICAL ROUTES:

Nano-sized particles can pass through biological membranes and penetrate even very small capillaries throughout the body (*e.g.,* pass through blood-brain and blood testes barriers).Size, morphology, and surface area are recognized as important determinants for toxicity of nanoparticles. Information on the toxicological implication of silver nanoparticles is limited. Toxicity of silver nanoparticles is mostly determined *in vitro* with particles ranging from approximately 1-100 nm. The *in vitro* and *in vivo* toxicity studies revealed that silver nanoparticles have the potential ability to cause chromosomal aberrations and DNA damage, to enter cells and cause cellular damage, and are capable of inducing proliferation arrest in cell lines of zebra fish. Limited health effects of the use of nanosilver particles in humans have been reported, such as argyria (a condition in which the skin becomes blue or bluish-grey colored because of improper exposure to chemical forms of element) which was appeared to occur only after intake of large amounts of colloidal silver particles (the suspension with nano-silver of different sizes). Several cross-sectional studies reported that argyria is the most frequent adverse outcome from exposure to silver nanoparticles. For instance, prolonged ingestion of colloidal silver can change the color of skin and cause blue-grey appearance on face (the symptoms of argyria). Silver nanoparticles can bind to different tissues (bind to proteins and enzymes in mammalian cells) and cause toxic effects, such as adhesive interactions with cellular membrane and production of highly reactive and toxic radicals like reactive oxygen species (ROS), which can cause inflammation and show intensive toxic effects on mitochondrial function. Potential target organs for nano-silver toxicity may involve liver and immune system. Accumulation and histopathological effects were seen in livers from rats systemically exposed to silver nanoparticles (10-15 nm). Furthermore, increased liver enzymes were reported in Sprague-Dawley rats after Twenty-eight-day oral exposure to nano-silver particles (60 nm). It was reported that silver-coated dressing acticoat caused raised liver enzymes and argyria-like symptoms in burn patients . The *in vitro* and *in vivo* studies revealed toxicity effects of silver nanoparticles on immune system, especially cytokine excretion. Application of 1% nano-silver cream (silver nanoparticles with size range of approximately 50 nm), inhibited DNB-induced allergic contact dermatitis, and accumulation in the spleen has also been reported. It has been suggested that silver nanoparticles are especially effective at inhibiting inflammations and may be used to treat immunologic and inflammatory diseases. Oral administration of silver nanoparticles (60 nm) to rats induced some local inflammatory effects. Moreover, in one *in vitro* study, toxicity effects of nano-silver particles on erythrocytes was reported, while an increase in red blood cells was seen after oral administration of silver

nanoparticles (60 nm), but not after inhalation of silver nanoparticles (15 nm). An *in vitro* study suggests that nano-silver particles may have cytotoxic effects on mammalian (mouse) spermatogonial germ line stem cells. Silver nanoparticles (10 µg ml-1 and above concentration) showed dramatic changes such as necrosis and apoptosis of the cells and at 5-10 µg ml-1 drastically reduced mitochondrial function and cell viability. It was reported that there is no evidence available to demonstrate that silver is a cause of neurotoxic damage even though silver deposits have been identified in the region of cutaneous nerves. The respiratory system seemed relatively unaffected by exposure to nano-silver *in vivo* in a 28 days study. However, cytotoxic effect of nano-silver particles on alveolar macrophages and alveolar epithelial cells was demonstrated, *in vitro*. In a 90 days inhalation study, decrease in lung functions, including tidal volume, minute volume and peak inspiration flow as well as inflammatory lesions in lung morphology and effects on inflammatory markers were reported performed a bone marrow micronucleus test as a part of a 28-day oral administration study in order to investigate genotoxic effects or carcinogenicity of exposure to silver nanoparticles. No significant genotoxic potential of oral exposure to silver nanoparticles (60 nm) was found in this study. An increase in (mostly local) malignant tumors was found following chronic subcutaneous administration of high doses colloidal silver [51].

CONCLUSION

The aim of the present review was focuses on the green synthesis of silver nanoparticles by using an endophytic fungi, its properties and general characterization techniques .The green synthesis methods are always cheap, reliable and nontoxic over the physical and chemical methods as it does not use any toxic chemicals and specific higher temperature and pressure. The green synthesis of silver nanoparticles using an endophytic fungi are eco friendly, safe and non toxic approach with great advantages as its produces large amount of nanoparticles as compared to bacteria by secreting large amount of protein which can directly translate to higher productivity of nanoparticles as well as can prevent the fast depletion of plant sources, instead of fungi provides protection and survival condition to their host plants. A brief discussion about the unique physical and chemical properties of silver nanoparticles has been illustrated such as optical, magnetic and catalytic properties etc. by which nanoparticles shows influence in their reactivity as compared to macrostructures. Furthermore this technique is a promising approach for synthesis of silver nanoparticles showing antimicrobial effect as illustrated.

REFERENCES

1. Devi, Lamabam Sophiya, and S. R. Joshi. Ultrastructures of silver nanoparticles biosynthesized using endophytic fungi. Journal of Microscopy and Ultrastructure 2015; 3(1): 29-37.
2. Wang, Ying, and N__ Herron. "Nanometer-sized semiconductor clusters: materials synthesis, quantum size effects, and photophysical properties." The Journal of Physical Chemistry 1991; 95(2): 525-532.
3. Prathna T.C.; Biomimetic Synthesis of Nanoparticles: Science, Technology & Applicability.www.intechopen.com
4. Kalaiselvam, M. "Extracellular Biosynthesis of Silver Nanoparticles by Endophytic Fungus Aspergillus terreus and its Anti dermatophytic Activity."International Journal of Pharmaceutical& Biological Archive 2013;4(3):481-487
5. Rai, Mahendra, Alka Yadav, and Aniket Gade. "Mycofabrication, mechanistic aspect and multifunctionality of metal nanoparticls–where are we? And where should we go." Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology 2010: 1343-1354.
6. Sunkar, Swetha., and C. Valli Nachiyar. "Endophytic fungi mediated extracellular silver nanoparticles as effective antibacterial agents." International Journal of Pharmacy and Pharmaceutical Science 5(2) ;2013: 95-100.
7. Williams, D., et al. "The appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies." Report for the European Commission Health and Consumer Protection Directorate General by the Scientific Committee on Emerging and Newly Identified Health Risks. Brussels ;2005.
8. Prabhu, Sukumaran, and Eldho K. Poulose. "Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical

applications, and toxicity effects."International Nano Letters 2(1) ;2012: 1-10.
9. Tran, Quang Huy, and Anh-Tuan Le. "Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives." Advances in Natural Sciences: Nanoscience and Nanotechnology 4(3) ;2013: 033001.
10. Vadlapudi, Varahalarao, and D. S. V. G. K. Kaladhar. "Review: green synthesis of silver and gold nanoparticles." Middle-East Journal of Scientific Research19.6 (2014): 834-842.
11. Panikkanvalappil R. Sajanlal,Theruvakkattil S . Sreeprasad, Akshaya K. Samal and Thalappil Pradeep. "Anisotropic nanomaterials: structure, growth, assembly, and functions.Nano Reviews 2011, 2: 5883 _1-5883_62 DOI: 10.3402/nano.v2i0.5883
12. Henglein, A. "Physicochemical properties of small metal particles in solution: "microelectrode" reactions, chemisorption, composite metal particles, and the atom-to-metal transition." J. Phys. Chem. 1993, 97(21), 5457-5471.
13. Burda, C.; Chen, X.; Narayanan, R.; El-Sayed, M. A. Chemistry and properties of nanocrystals of different shapes. Chem. Rev. 2005, 105(4), 1025-1102.
14. Kelly, K.L.; Coronado, E.; Zhao, L.L.; Schatz, G.C. "The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment." J.Phys. Chem. B107. 2002, 34(16) , 668-677.
15. Liz-Marzán, L. M.; Nanometals: formation and color, Materials today 2004; 7, 26-3.
16. Treguer-Delapierre M, Majimel J, Mornet S, Duguet E, Ravaine S. Synthesis of non-spherical gold nanoparticles. Gold Bull 2008; 41:195–207
17. Lee I, Morales R, Albiter MA, Zaera F. Synthesis of heterogeneous catalysts with well shaped platinum particles to control reaction selectivity. Proc Natl Acad Sci USA 2008; 105: 15241-6.
18. W.H. Qi, M.P. Wang. Size and shape dependent melting temperature of metallic nanoparticles. Materials Chemistry and Physics 2004; 88: 280–284
19. Kruis, F.; Fissan, H. & Rellinghaus, B.. Sintering and evaporation characteristics of gas-phase synthesis of size-selected PbS nanoparticles. Mater Sci Eng B, 2000; 69:329-324
20. Chen, Meng, et al. "Silver nanoparticles capped by oleylamine: formation, growth, and self-organization." Langmuir 23.10 (2007): 5296-5304.
21. Ahmad, A.M., P.; Senapati, S.; Mandal, D.; Khan, M.I.; Kumar, R. & Sastry, M., "Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum". Colloids and Surfaces B: Biointerfaces, 2003. 28: 313-318.
22. Ankamwar, B.D., C.; Ahmad, A. & Sastry, M., "Biosynthesis of gold and silver nanoparticles using Emblica officinalis fruit extract, their phase transfer and transmetallation in an organic solution". J Nanosci Nanotechnol, 2005; 5(10):1665-71
23. Jha, A., Prasad, K, Prasad, K, Kulkarni, AR, "Plant system: nature's nanofactory". Colloids Surf. B Biointerfaces, 2009. 73: 219-223.
24. Rauwel, Protima, et al. "A Review on the green synthesis of silver nanoparticles and their morphologies studied via TEM." Advances in Materials Science and Engineering 2015.
25. Haefeli, Cathierine., Christopher. Franklin, and Hardy. "Plasmid-determined silver resistance in Pseudomonas stutzeri isolated from a silver mine." Journal of bacteriology 1984;158(1): 389-392.
26. Husseiny, M. I., et al. "Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa." Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 2007;67(3): 1003-1006.
27. Mohanpuria, P., Rana, KN, Yadav, SK, "Biosynthesis of nanoparticles: technological concepts and future applications". J. Nanopart. Res., 2008. 10: 507-517.
28. Mukherjee, Priyabrata, et al. "Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis." Nano Letters 2001;1(10): 515-519
29. Ahmad, Absar, et al. "Extracellular biosynthesis of silver nanoparticles using the fungus Fusarium oxysporum." Colloids and Surfaces B: Biointerfaces 2003;28(4): 313-318.
30. Tan RX, Zou WX: Endophytes: a rich source of functional metabolites.Nat Prod Rep 2001, 18:448–459.
31. R.X. Tan, W.X. Zou, "Endophytes: a Rich Source of Functional Metabolites", Nat. Prod. Rep., 2001;18, 448–459,
32. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mole Biol Rev, 2003; 67: 491–502.
33. Compounds.5. Strobel, G. and Daisy, B., Microbiol. Mol. Biol. Rev., 2003, 67, 491–

502.6. Strobel, G. A. et al., Microbiology, 1999, 145, 1919–1926.
34. Schulz B, Mmert R, Dammann AK, Aust U, Strack D. The endophyte-host interaction: a balanced antagonism. Mycol. Res, 1999; 103: 1275–1283.
35. Parasharu K, S.a.S.A., "Bioinspired Synthesis of Silver Nanoparticles". Digest Journal of Nanomaterials and Biostructures, 2009. 4: 159-166.
36. .Kashyap PL, Kumar S, Srivastava AK, Sharma AK. Myconanotechnology in agriculture: a perspective. World J Microbiol Biotechnol, 2013; 29(2): 191-207
37. Gade A, Bonde PP, Ingle AP, Marcato P, Duran N, Rai MK. Exploitation of Aspergillus niger for synthesis of silver nanoparticles. Journal of Bio based Materials and Bioenergy, 2008; 2(3): 1–5.
38. Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, Khan MI. Nitrate reductase-mediated synthesis of silver nanoparticles from AgNO3. Biotechnol Lett, 2007a; 29(3): 439–445.
39. UV/Vis/IR spectroscopy analysis of nanoparticles-, V 1.1; nano Composix. September2012. http://50.87.149.212/sites/default/files/nanoComposix%20Guidelines%20for%20UV-vis%20Analysis.pdf
40. Transmission Electron Microscopy analysis of nanoparticles-, V 1.1; nano Composix September 2012. http://50.87.149.212/sites/default/files/nanoComposix%20Guidelines%20for%20TEM%20Analysis.pdf
41. X-ray diffraction: a powerful method of characterizing nanomaterials; Recent Research in Science and Technology 2012, 4(8): 77-79.
42. Nameirakpam, Nirjanta Devi, Shankar P. Dheeban, and S. Sutha. "Biomimetic synthesis of silver nanoparticles from an endophytic fungus and their antimicrobial efficacy". International Journal of Biomedical and Advance Research 2012;3(5): 409-415.
43. Kl, Niraimathi, et al. "Green synthesis and characterization of silver nanoparticles from aqueous extract of basella alba and their in-vitro antioxidant potentials." International Journal of Pharmacy and Pharmaceutical Sciences 2014:6(10):393-396.
44. Puchalski, M., et al. The applicability of global and surface sensitive techniques to characterization of silver nanoparticles for Ink-Jet printing technology.63-79. INTECH Open Access Publisher, 2010.] http://cdn.intechopen.com/pdfs-wm/9724.pdf.
45. Prabhu, Sukumaran, and Eldho K. Poulose. "Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. "International Nano Letters 2012;2(1): 1-10.
46. Tran, Q. H., & Le, A. T. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Advances in Natural Sciences: Nanoscience and Nanotechnology 2013: 4(3), 033001
47. Cho et al. 2005; Dura´n et al. Silver nanoparticles show potential antimicrobial effects against infectious organisms, including Escherichia coli, Bacillus subtilis, Vibria cholera, Pseudomonas aeruginosa, Syphillis typhus, and S. aureus 2007. 63-79
48. De Gusseme B, Hennebel T, Christiaens E, Saveyn H Verbeken K, Fitts J P, Boon N and Verstraete W Virus disinfection in water by biogenic silver immobilized in polyvinylidene fluoride membranes. Water Res. 2011, 45(4):1856-64.
49. Yoon K Y, Byeon J H, Park C W and Hwang J Antimicrobial effect of silver particles on bacterial contamination of activated carbon fibers. Environ. Sci. Technol 2008 Feb 15;42(4):1251-1255.
50. Hussain, S.M.; Hess, K.L.; Gearhart, J.M.; Geiss, K.T. & Schlager, J.J. In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol in vitro, 2005;19:.975-983.
51. http://cime.epfl.ch/page-26783-en.html