A DISSERTATION ON

# GREEN SYNTHESIS OF GOLD NANOPARTICLE FROM FUNGUS

SUBMITTED

TO THE

DEPARTMENT OF BIOSCIENCES

INTEGRAL UNIVERSITY, LUCKNOW.

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FOR THE

DEGREE OF MASTER OF SCIENCE

IN MICROBIOLOGY

BY

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# DECLARATION

I hereby declare that the present work on **"Green synthesis of gold nanoparticles from fungus"** is a record of original work done by me under the guidance of march to June 2022, at Integral University Lucknow. All the data which were provided in this were through our own original work.

I also declare that not any part of this thesis has previously been submitted to my university or any examining body for acquiring any diploma or degree.

**Place: Integral University, Lucknow**

**Date-:25/06/2022**

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# CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Miss Shalini Tiwari** during the months March-June 2022 reported in the present thesis was under my guidance and supervision The results reported by him are genuine and script of the thesis has been written by the candidate herself. The thesis entitled **"Green synthesis of gold nanoparticles from fungus"** is therefore, being forwarded for the acceptance in partial fulfillment of the requirements for the award of the degree of Master of Science in Microbiology, Department of Biosciences, Integral University, Lucknow (U.P).


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## To WHOM IT MAY CONCERNS

This is to certify that **Shalini Tiwari** a student of M.Sc Microbiology (IV Semester) Integral University has completed fourth months dissertation work entitled **"GREEN SYNTHESIS OF NANOPARTICAL FROM FUNGUS''** successfully. She has completed this work from March to June 2022 under the supervision of **Dr. Arshi Siddiqui** (Assistant Professor, Department of Biosciences).and co supervision **Dr. Uzma Afaq** The dissertation was a compulsory part for the award of her M.Sc degree in Microbiology.

I wish her good luck and bright future.

**(Dr. Snober S. Mir)**

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# INTRODUCTION

Nanoparticles are solid colloidal particles ranging from 1-1000 nm in size and, they consist of macromolecular, materials in which the active ingredient is dissolved, entrapped, encapsulated or absorbed structure and size are 0D,1D,2D,3D Fullerene carbon Nanotube, Graphene, Graphite. Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties. Some of them are carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles. Nanoparticles are created the gas phase by producing a vapor of the product material using chemical or physical means. The production of the initial nanoparticles, which can be in a liquid or solid state, takes place via homogeneous nucleation. Naturally occurring nanoparticles can be found in volcanic ash, ocean spray, fine sand and dust, and even biological matter. Synthetic nanoparticles are equally, if not more diverse than their naturally. nanoparticle comes in the environment by natural processes or can be manufactured by various physical, chemical, and biological methods.

Physicist **Richard Feynman**, the father of nanotechnology. Nanoscience and nanotechnology are the study and application of extremely small things and can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering.

Green synthesis of nanoparticles rapid progressions and technological innovations in the field of science and technology have generated immense interest among the research community across the globe to explore novel aspects of nanotechnology. Nanotechnology can be defined as the manipulation of matter such that any one of its dimensions falls in nanoscale range (*100nm*).

Nanotechnology is the branch of science which deals with the examination of materials in nano range, generally between 1 to 100 nm. It is a science that works at the nanoscale and gives various focal points to the diverse fields of science like dentistry, pharmaceuticals and bioengineering (Gour & jain *et al.,* 2019).

Green chemistry approach is significant for the future prospect of nanomaterials. Thi s area of nanoscience should culminate in the development of safe, eco-friendly NPs and should have wide acceptance in the nanotechnology (Varma *et al*., 2019).

Solventreducing operators utilized for the reduction of the NPs have great effect on morphology of incorporated particles like their size, physicochemical properties, ssha pe and this morphology impacts on the utilization of NPs. "Top down" and "bottom up " are the two unique methodologies for the amalgamation of NPs. In bottom. approac h, suitable bulk material is broken down into smaller fine particles by size reduction u sing various techniques like grinding, milling, sputtering, thermal/laser ablation, while in bottom.

Top approach, NPs are synthesized using chemical and biological methods by self-assembly of atoms to new nuclei, which grow into nanosize particles while the "botto m-up" methods include chemical reduction, electrochemical methods and decomposition (Mathura *et al.,* 2018).

Several advances report development of metal NPs that preclude surfactant and an y other chemicals (Nadagouda & Varma *et al*.,2008).

The green synthesis from microbes, parasites, plants and plants extracts and so on are reviewed in the consequent segments of this review.

Green methods of synthesis are significantly attractive because of their potential to reduce the toxicity of NPs. Accordingly, the use of vitamins, amino acids, plants extracts is being greatly popularized nowadays (Baruwati *et al.,*2009). Rapid progressions and technological innovations in the field of science and technology have generated immense interest among the research community across the globe to explore novel aspects of nanotechnology. Nanotechnology can be defined as the manipulation of matter such that any one of its dimensions falls in the (Kaushik et al., 2010). Particles generated at the nanoscale have varied unique properties (optical, magnetic, electrical, etc.) because of their very large specific surface area, high surface energy, and quantum confine (Hussain & Singh *et al,.*2016). Due to these exclusive physicochemical properties, nanoparticles find endless applications in medicine (Azzazy &Samir *et al.,* 2012). Cosmetics, electronics the food industry, and the chemical industry Metallic nanoparticles can be generated through various routes comprised of physical, chemical, and biological pathways (Iravan & zolfaghari *et al.,* 2014).

These various physicochemical and biological pathways for nanoparticle synthesis fall under two distinct categories: a top-down approach and a bottom-up approach. The top-down approach involves processes in which nanoparticles are generated via size reduction, whereas in the bottom-up approach, nanoparticles are generated from small entities such as atoms and molecules (Sepeur *et al.,* 2008). The commonly used physical methods for the synthesis of nanoparticles large (Mafune &Sawabe*, &* Tseng et al., 2016) inert gas condensation electric arc discharge (Tseng 2016).and the radiofrequency (RF) plasma method (Hiragino Tanaka& Fujita *et al.,* 2016). these physical methods require a lot of time to achieve thermal stability, consuming a lot of energy while raising the environmental temperature around the source material, along with occupying large spaces in the case of tube furnaces (kawasakhi & Nishimura *et al.,*2006). The physical synthesis pathway is not suitable for the generation of nanoparticles. The major disadvantage with the chemical synthesis of nanoparticles is that it employs harsh reducing agents such as sodium borohydride, sodium citrate, etc., and organic solvents (Tarasenk*o et al*., 2006). These chemical reagents pose toxicity issues along with environmental (Pal *et al*.,2007). Due to the aforesaid reasons, the biological synthesis pathways are preferred over physical and chemical synthesis methods for the generation of nanoparticles. The biological synthesis method for the generation of nanoparticles involves the use of bacteria & (Ayano *et al.,*2014). algae (Faraz & Pandey *et al*, 2015).

and plants (Duan *et al.,* 2015). In addition to being slow, the pathogenicity issue and the maintenance of large-scale cultures are major drawbacks associated with the use of microorganisms for the generation of nanoparticles. Green synthesis of nanoparticles involves the use of plant or plant parts for the bio reduction of metal ions into their elemental form in the size range 1–100nm.The process of green synthesis is more efficient, simpler, and economical, and can easily be scaled up to perform larger operations (Iravani *et al.,* 2014) .Also, there is no need to maintain large-scale cultures, and the process does not pose a biohazard problem as in the case of microorganism-mediated synthesis of nanoparticles (Iravani &Banerjee *et al*.,2013) .oxide .

Nanotechnology provides a platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in biomarkers, diagnostics, cell labelling, contrast agents for biological imaging, drug delivery system an antimicrobial agents and nano drugs for treatment of various disease (Sing *et al.,* 2011). Nanotechnology is a multidisciplinary field that covers wide range of physical, chemical, electrical, biological and electronics engineering. Nanotechnology is expected to be the basis of many main technological innovations in the 21st century. Research and development in this field is growing rapidly throughout the world. For the synthesis of nanoparticles, a number of chemical methods exist in the literature all these protocols involve toxic chemicals, which have been a matter of great concern for environmental reasons. Consequently, researchers in the field of nanoscale material synthesis and assembly have been eagerly looking at biological systems for an alternative (Ahmad *et al.,*2003). Microorganisms such as bacteria, yeast, fungi and actinomycetes have been described for the formation of nanoparticles and their applications (Sastry *et al*., 2003). The metabolic activity of microorganisms can lead to precipitation of nanoparticles in external environment of a cell, the fungi being extremely good candidates for synthesis of nanoparticles. The extracellular synthesis of silver and gold nanoparticles by the fungus Colletotrichum sp. or Aspergillus fumigatus has been reported. The mass production fungi i easy, for synthesis of nanoparticles. Due to their physiochemical properties, silver nanoparticles have been widely employed and currently used as anti-bacterial agents in food storage, textile and health industries and health industries, for bio labeling and biosensors. The antimicrobial activity of silver nanoparticles has now been well established and they possess inflammatory antifungal activity. (Prakash *et al*.,2012). Filamentous fungi are more advantageous over the bacteria and algae because fungi having fungal mycelia mesh which can withstand flow pressure and agitation and other conditions in the bioreactors. The fungi like ISSN: 2319-9490 International Journal of Current Research in Life Sciences Vol. 07, No. 01, pp.788-791, January2018 Fusarium solani USM3799, F. oxysporium, Aspergillus niger, Coriolus versicolor are capable of synthesizing silver nanoparticles (Saha *et al.,* 2011).

Infections caused by Aspergillus species have grown in importance in recent years. This probably results from a higher number of patients being at risk, including transplant recipients, neutropenic individuals, allergic patients and those treated with corticosteroids or other immunosuppressive regimens. Despite a better understanding of the epidemiology of Aspergillus infections, important diagnostic limitations persist. Accordingly, the mortality for invasive aspergillosis remains very high. As most of the Aspergillus infections are caused by *A. fumigatus*, the majority of studies have focused on this species, and our understanding of other Aspergillus species is far from satisfactory*. Aspergillus flavus* is the second leading cause of invasive and non-invasive *aspergillosis*. In addition, it is the main *Aspergillus species* infecting insects, and it is also able to cause diseases in economically important crops, such as maize and peanuts, and to produce potent mycotoxins. Curiously, some Aspergillus syndromes are rarely associated with *A. flavus.* The aim of this review is to summarize the available data comparing the pathogenicity of these two medically important thermotolerant fungi*, A. fumigatus and A. flavus*. In addition, clinical syndromes particularly associated with *A. flavus* are presented.

Gold nanoparticles (GNPs) have gained attention in recent years due to their emerging diverse applications in the field of biomedicine, bioimaging, biosensors, and biolabeling, etc.  There has been a great deal of interest in developing versatile protocols to synthesize GNPs with controlled properties. Conventional approaches used to synthesize GNPs are cost intensive and not environment-friendly. Further, use of chemical substrates during chemical synthesis of nanoparticles restrict use in clinical and pharma applications also. Hence, development of environment friendly, biocompatible non-toxic protocols has been the focus in the field of nanostructure synthesis. Biosynthesis of nanoparticles using microbes could be a promising alternative. Marine microbes are less explored despite the fact that researches on nanobiotechnology in future may increasingly depend on marine microbes having ability to grow under extreme conditions. Considering  the developing global scenario. it has been suggested that initiatives should be taken for exploitation of marine microorganism in  the area of nanobiotechnology  Among microbes, unique features of fungi like their greater growth capacity, reach by virtue of mycelial branching, greater potential to produce a number of enzymes, and ability to accumulate various metals, besides having ability to grow under extreme conditions make them more efficient entities for such purposes. Fungal isolates having remarkable metal removal efficiency have been screened out from marine habitats, however, scant information is available on exploitation of marine-derived fungi for biosynthesis of metal nanoparticles.

Especially, to the best of our knowledge, so far only one report exists on biosynthesis of GNPs using marine derived filamentous fungi, which is from our laboratory. Keeping this in view, an effort has been made to explore potential of a marine-derived fungus to produce spherical monodisperse GNPs. The data revealed that the test isolate could biosynthesize GNPs with an average size of 10 nm.

The conventional methods for the production of NPs are expensive, toxic, and non-environment friendly. To overcome these problems, researchers have found the precise green routes, i.e., the naturally occurring sources and their products that can be used for the synthesis of NPs. Green synthesis can be categorized as: (a) utilization of microorganisms like fungi, yeasts (eukaryotes), bacteria, and actinomycetes (prokaryotes), (b) use of plants and plant extracts (c) use of templates like membranes, viruses DNA, and diatoms. The green synthesis via bacteria, fungi, plants, and plant extracts are described in the further sections.

Fungi have potential for the synthesis of metallic NPs due to metal bioaccumulation capacity and their tolerance, high binding capacity, and intracellular uptake like bacteria that are easy to handle in a research facility as compare to bacteria (Sastry *et al.,*2003). Fungi can be used through different methods for the synthesis of NPs, in which fungi secrete enormous enzymes which are utilized to reduce $AgNO_3$ solution (Mandal *et al.,*2006). A schematic representation is shown in. The extracellular synthesis of Ag-NPs by utilizing *F. oxysporum* and its antibacterial effect on textile fabrics is studied by (Durán *et al.,*2007) reported that mono-disperse Ag-NPs can be synthesized by using fungus *Aspergillus flavus* and average size of the NPs observed in the range of 8.92 ± .61 is measured by Transmission Electron Microscopy (TEM). The extracellular synthesis of Ag-NPs using fungus *Cladosporium cladosporioides* is observed by Balaji et al. and the size of NPs is observed by TEM in the range of 10–100 nm (Balaji *et al.,*2009). In another method (kathiresan *et al.,*2009). illustrated *in vitro* synthesis of Ag-NPs using $AgNO_3$ as a substrate and *Penicillium fellutanum* isolated from coastal mangrove sediment. Ahmad *et al*.,2003). studied that aqueous Ag ions when exposed to the *Fusarium oxysporum* are reduced in solution by an enzymatic process, prompting the formation of highly stable Ag hydrosol. The NPs are in the size range 5–15 nm and are stabilized in solution by protein-secreted fungus. The extracellular synthesis of mono-dispersed Ag-NPs is achieved by Bhainsa

& D’Souza *et al,* 2006) using *Aspergillus fumigatus* at quick synthesis rate. In another method, spherical Ag-NPs are synthesized by using *Aspergillus terrus* with an average size of 1–20 nm.

# REVIEW OF LITERATURE

*Aspergillus niger* is a Fungus and one of the most common species of the genus Aspergillus causes a disease called "black mold" on certain fruits and vegetables such as grapes, apricots, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in Soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stacy botr (species of which have also been called "black mold").

Some strains of *A. niger* have been reported to produces mycotoxins called ochratoxins other sources disagree, claiming this report is based upon misidentification of the fungal species. Recent evidence suggests some true *A. niger* strains do produce ochratoxin A It also produces the isoflavone Orobol.

*Aspergillus. Niger* is less a like disease causing to a directly infection to a human some other *Aspergillus species*. In extremely rare instances, humans may become ill, but this is due to a serious lung disease, aspergillosis that can occur. Aspergillosis is, in particular, frequent among horticulture workers who inhale peat dust, which can be rich in *Aspergillus* spores. The fungus has also been found in the mummies other *Aspergillus* species. In extremely rare instances, humans may become ill, but this is due to a serious lung disease, aspergillosis that can occur. Aspergillosis is, in particular, frequent among horticulture workers who inhale peat dust, which can be rich in *Aspergillus* spores. The fungus has also been found in the mummies of ancient Egyptian tombs and can be inhaled when they are disturbed Handwerk & Braine *et al.,*2005)*. A. niger* is one of the most common causes of otomycosis (fungal ear infections), which can cause pain, temporary hearing loss, and, only in severe *Aspergillus niger* is cultured for the industrial production of many substances. (Nai & Meyer *et al*.,2018) various strains of *A. niger* are used in the industrial preparation of Citric acid (E330) and Gluconic acid (E574) and have been assessed as acceptable for daily intake by the World Health Organization. *A. niger* fermentation is "generally recognized as safe" (GRAS) by the United. *A. niger* is also considered as a potential alternative source of natural food grade pigments.

devised various processes for the synthesis of nano and micro length scaled inorganic materials which have contributed in the development of relatively new and

| | |
|---|---|
| Kingdom | *fungi* |
| Division | *Ascomycota* |
| Class | *Eurotiomycetes* |
| Order | *Eurotiales* |
| Family | *Trichocomaceae* |
| Genus | *Aspergilus* |
| Species | ***A.niger*** |

largely unexplored area of research based on the biosynthesis of the nanomaterials. Synthesis using bio-organisms is compatible with the green chemistry principles. “Green synthesis” of nanoparticles makes use of environment friendly, non-toxic and safe reagents. (Banse &Ledwani *et al,.*2016). Nanoparticles synthesized using biological techniques or green technology have diverse natures, with greater stability and appropriate dimensions since they are synthesized using a one-step procedure. Nanoparticles can be synthesized using a variety of methods including chemical, physical, biological, and hybrid techniques laser desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epitasis a Similarly, chemical methods are used to synthesize NPs by electro deposition, sol–gel process, chemical solution deposition, chemical vapour position soft chemical method, Langmuir Blodgett method, catalytic route, hydrolysis co-precipitation method and wet chemical method. Chemical and Physical methods have been using high radiation and highly concentrated reductants and stabilizing agents that are harmful for the environmental and to human health. Hence, biological synthesis of nanoparticles is a single step bio-reduction method and less energy is used to synthesize eco- friendly.

## Synthesis of Ag-NPs by using fungi

The fungal mediated green chemistry approach towards the fabrication of NPs has many advantages. This includes easy and simple scale up method, economic viability, easy downstream processing and biomass handling, and recovery of large surface area with optimum growth of mycelia. It has been observed that most of the fungal genera are coupled with the synthesis of Ag-NPs either intracellularly or extracellularly showing the onset of deep brown coloration. Aqueous Ag ions exposed to Fusarium oxysporum lead to the fabrication of extremely stable Ag hydrosol. The particles are stabilized in solution by the proteins excreted through the fungus, Extracellular biosynthesis of Ag-NPs in the 5-25 nm range using Aspergillus fumigatus is found to be quite fast and manifested the production of dense fungal biomass. White rot fungus, scientifically known as Phaenerochaete chrysosporium has also been used for biomimetics of AgNPs (Vigneswaran& Balasubramanya *et al*.,2006). Aspergillus flavus has been challenged with AgNO3 solution it accumulated Ag-NPs (Gade *et.al.,*2008). on the surface of its cell wall. Very recently Li et al. demonstrated a method for AgNPs synthesis by reduction of aqueous Ag+ with the culture supernatants of *Aspergillus terreus* at room temperature. The synthesized Ag-NPs were poly dispersed spherical particles ranging in size from 1 to 20 nm and stabilized in the solution. Reduced NADH was found to be an important reducing agent for the biosynthesis, and the formation of Ag-NPs might be an enzyme-mediated extracellular reaction process. Their antimicrobial potential was systematically evaluated and found that they could efficiently inhibit various pathogenic organisms, including bacteria and fungi Qian (Giuan & wang *et al.,*2011).

Fungi have potential for the synthesis of metallic NPs due to metal bioaccumulation capacity and their tolerance, high binding capacity, and intracellular uptake like bacteria that are easy to handle in a research facility as compare to bacteria (Sastry *et al.,* 2003). Fungi can be used through different methods for the synthesis of NPs, in which fungi secrete enormous enzymes which are utilized to reduce $AgNO_3$ solution (Mandal *et al*., (2006). A schematic representation is shown in the extracellular synthesis of Ag-NPs by utilizing *F. oxysporum* and its antibacterial effect on textile fabrics is studied by (Durán *et al*., (2007). Vigneshwaran reported that mono-disperse Ag-NPs can be synthesized by using fungus *Aspergillus flavus* and average size of the NPs observed in the range of $8.92 \pm 1.61$ is measured by Transmission Electron Microscopy (TEM). The extracellular synthesis of Ag-NPs using fungus *Cladosporium cladosporioides* is observed by Balaji et al. and the size of NPs is observed by TEM in the range of 10–100 nm (Balaji *et al*., 2009). In another method (Kathiresan *et al.,* (2009) illustrated *in vitro* synthesis of Ag-NPs using $AgNO_3$ as a substrate and *Penicillium fellutanum* isolated from coastal mangrove sediment (Ahmad *et al.,* 2006). studied that aqueous Ag ions when exposed to the *Fusarium oxysporum* are reduced in solution by an enzymatic process, prompting the formation of highly stable Ag hydrosol. The NPs are in the size range 5–15 nm and are stabilized in solution by protein-secreted fungus. The extracellular synthesis of mono-dispersed Ag-NPs is achieved by (Bhain *et al.,* 2011*). Aspergillus fumigatus* at quick synthesis rate. In another method, spherical Ag-NPs are synthesized by using *Aspergillus terreus* with an average size of 1–20 nm.

Traditional physical and chemical approaches make use of toxic reagents that are hazardous to the environment. They also require expensive, sophisticated instrumentation and these processes are usually conducted under drastic conditions that involve the use of elevated temperatures, very high/low pressure and expensive reagent. (Chowdhury & Naskar, *et al.,*2015). These here is a growing need to develop strategies that are cost-effective and more importantly not detrimental to the environment. The use of biological materials in the synthesis of nanoparticles may be considered as one such eco-friendly, “green” alternative. These approaches have the added advantage of using benign solvents and biological materials are usually capable of performing the dual function of reducing as well as acting as a

capping agent in the synthesis and stabilization of the nanoparticles. (Sreenivasan & Balaji, *et al*.,2014).

## Fungal mediated synthesis of copper oxide nanoparticles-:

Over the last few years, various fungi species have been used for the synthesis of copper oxide and other metal nanoparticles (Waris *et al.,* (2020). As compared to other microorganisms, fungi possess high potential for the synthesis of nanoparticles in many ways. Fungi bear agitation, flow pressure & other conditions in the bioreactor or any other growth chamber compared to bacteria. Cell-free extracts of microorganisms act as reducing, catalysis, or capping agents for the biogenic fabrication of nanoparticles. A well-known fungi Trichoderma species produces different types of bioactive metabolites like pyrones, polyketides, terpenes, diketopiperazine, glycolipids, and enzymes, however, they are not involved in the synthesis of copper oxide nanoparticles. Fungi synthesize nanoparticles by two main pathways; intracellular and extracellular pathways. The size of nanoparticles synthesized inside the fungal species could be smaller, with good dispersity and well dimensions compared to the extracellular route Fabrication of nanoparticles through the extracellular pathway has many advantages. The synthesized nanoparticles could be free of cell components. Mostly, the extracellular pathway of fungi has been exploited for the synthesis of nanoparticles as the fungi secret different types of metabolites that act as reducing and stabilizing agents for the synthesis of nanoparticle. Different strains of fungi have been exploited for the synthesis of metal oxide nanoparticles, including copper oxide nanoparticles. Green mediated copper oxide nanoparticles using fungal strain Trichoderma have been 183 produced. Cu (NO3)2.3H2O salt was added to mycelial-free water extract and stirred at 40 °C 184 overnight in the dark followed by heating for 3 hr. at 75-80 °C temperature. The solution changed 185 the color that indicated the production of copper oxide nanoparticles. Finally, nanoparticles were washed and characterized by UV-visible, XRD, HRSEM, PSA, XPS, TEM, EXD, and FTIR for size, morphology, texture, crystallinity, purity, functional groups, and chemical analysis. It has been concluded from characterizations that the synthesized copper oxide nanoparticles were nm an average size with spherical morphology. FTIR analysis detected amide and secondary aromatic metabolites that indicated these functional groups were responsible for the reducing, capping, and stabilizing copper oxide nanoparticles (Cuevas *et al.,* 2015). also reported the fabrication of copper oxide nanoparticles using white-rot fungus. The mycelium193 free fungal extract was isolated and exposed to the solution containing different copper salts CuCl2, Cu (NO3)2, and CuSO4, kept in the shaker (100 rpm) for seven days in the dark at 25 °C 195 temperature. The solution changed the color to dark brown that indicated the formation of copper 196 oxide nanoparticles. The nanoparticles were then washed and analyzed their size, shape, and chemical properties by different techniques such as UV-vis, XRD, TEM, and FTIR. The synthesized copper oxide nanoparticles were spherical shaped and the dimension of 5-20 nm Bio-inspired fabrication of copper oxide nanoparticles using *Penicillium chrysogenum* has been reported by El-(Batal *et al.,* (2019). The characterizations for identification were confirmed by UV, FT Viz IR, XRD, DLS, TEM, SEM, and EDX. The authors reported that the biogenic copper oxide nanoparticles were spherical morphology and a mean diameter of 9.7 nm. FTIR analysis also revealed the presence of an amide functional group that was involved in the reduction and stabilization of copper oxide nanoparticle. The antimicrobial activity against different plant pathogenic bacteria and fungi have also been investigated, and results showed that the green synthesized copper nanoparticles were highly effective against Fusarium *oxysporum* followed by *Alternaria solani*, and Aspergillus niger and were active against various bacteria such as *Ralstonia* solanacearum and *Erwinia amylovora* provides some examples of fungal mediated synthesized copper oxide nanoparticles.

## Components of synthesis of NPs-

Distinctive natural specialists respond contrastingly with metal particles prompting the arrangement of NPs so the exact instrument for synthesis combination through organic methods shall have to be considered. For the most part, NPs are biosynthesized when the micro-organisms, plant extracts snatch target particles from their condition and afterward transform the metal particles into the NPs through the catalysts produced by the cells itself. It can be characterized into intra-cellular and extra-cellular amalgamation depending upon the area where NPs are framed.

Methodologies of NPs synthesis Chemical approach. In the chemical approach, the main components are the metallic precursors, stabilizing agents and reducing agents (inorganic and organic both). Reducing agents such as sodium citrate, ascorbate, sodium borohydride ($NaBH_4$), elemental hydrogen, polyol process, tollens reagent N N-dimethyl for mamide (DMF) and poly (ethylene glycol) block copolymers are used.

Physical approach. Physical approach for synthesizing NPs is mainly “top-down” approach in which the material is reduced in size by various physical approaches like ultra-sonication, microwave (MW) irradiation, electrochemical method etc. In this approach, a tube heater is utilized at barometrical weight for integrating NPs by evaporation condensation. Evaporation condensation and laser removal are the most essential physical methodologies. The source material inside a pontoon focused at the heater is vaporized into a bearer gas. Utilizing this dissipation build-up procedure different NPs of Ag, Au, PbS and Cd have been synthesized and reported already.

## Green approach for synthesis of NPs

Traditional methods are used from past many years but researches have proved that the green methods are more effective for the generation of NPs with the advantage of less chances of failure, low cost and ease of characterization Physical and chemical approaches of synthesizing NPs have posed several stresses on environment due to their toxic metabolites. Plant-based synthesis of NPs is certainly not a troublesome procedure, a metal salt is synthesized with plant extract and the response is completed in minutes to couple of hours at typical room temperature. This strategy has attracted much more attention amid the most recent decade particularly for silver (Ag) and gold (Au) NPs, which are more secure as contrasted with other metallic NPs. Generation of NPs from green techniques can be scaled up effortlessly and they are fiscally smart too. In light of their exceptional properties the greenly orchestrated NPs are currently favoured over the traditionally delivered NPs. Use of more chemicals, which are harmful and toxic for human health and environment, could increase the particle reactivity and Components of synthesis of NPs.

Distinctive natural specialists respond contrastingly with metal particles prompting the arrangement of NPs so the exact instrument for synthesis combination through organic methods shall have to be considered. For the most part, NPs are biosynthesized when the micro-organisms, plant extracts snatch target particles from their condition and afterward transform the metal particles into the NPs through the catalysts produced by the cells itself. It can be characterized into intra-cellular and extra-cellular amalgamation depending upon the area where NPs are framed.

## Systems for green synthesis of NPs

### Green synthesis from enzymes

Well-defined structure and available purity of enzymes makes them preferable for green method of synthesis, eAg NPs were utilized to be combined by an enzyme induced growth process on strong substrates in NPs synthesis. The enzymes were incorporated in polymer multilayer-assembled membranes through electrostatic interactions to develop the direct and "green" synthesis of bimetallic Fe/Pd particles in a membrane domain The generation of Au NPs utilizing extracellular amylase for the decrease of AuCl4 with the maintenance of enzymatic movement in the complex has been accounted for. Reaction surface strategy and central composite rotary design (CCRD) were utilized to upgrade a fermentation medium for the generation of a-amylase by Bacillus licheniformis at pH 8. A sulphatic reductase enzyme purified from the E. coli by using ion exchange chromatography was used to develop a cell-free extract for the Au NPs synthesis having antifungal activity against human pathogenic fungi. The nanoparticles (NPs) can be synthesized from agro-waste like Cocos nucifera coir, corn cob, fruit seeds and peels, wheat and rice bran, palm oil, etc. These compounds are rich in biomolecules like flavonoids, phenolic and proteins that could act as reductive agent for the synthesis of NPs. In a reported method of reduction of NPs with beet juice the authors found that on decreasing the amounts of beet juice, larger size Ag NPs were obtained, which also showed much greater catalytic activity and stability than those prepared with NaBH4 for the transformation of 4-nitrophenol to 4-aminophenol Green tea extracts were used to get bimetallic NPs of Fe/Pd for the first time as the extracts of green tea can act as both reductive as well as capping agent also Au NPs functionalized mediated with a redox enzyme can perform as an electron transmitter between the biocatalyst and the electrode and accordingly give a hybrid electrically dynamic biomaterial that can be utilized in different sensors applications.

## Green synthesis from vitamins

Green combination of Ag and palladium nano-spheres, nanowires and nano-rods by utilizing vitamin B2 (as reducing and capping agents) has been reported. The vitamin B2 is used as the reducing agent for the synthesis of the nanowires and nanorods. This is a unique approach, in the field of green nanotechnology that suggests the use of natural agents in advancement of this field, for example their impact on different tumors cells. Ascorbic acid is used as capping and reducing agent along with the chitosan as stabilizing agent due to the property of chitosan bonding with metal ions, NPs concentration is directly dependent on chitosan concentration used A simple method of developing NPs of uniform sizes produced by using ascorbic acid as the reducing.

and capping material is reported. Water-soluble anti-oxidative agents like ascorbic acid further seem to be responsible for the reduction of Ag NPs in *Disodium triflorum*. During glycolysis, plants produce a large amount of Hþ ions along with NAD and acts as a strong reducing agent; this seems to be beneficial in the formation of Ag NPs.

## Microwave-assisted synthesis

Nanowires, tubes and dendrites can be produced by altering the parameters like surfactants and metallic precursors and solvent. Spherical shaped nanomaterials can also be produced by this method Carboxyl methyl cellulose sodium is utilized as reducing and capping specialist for Ag NPs synthesis. Contrasted with general heating treatment, MW union is supportive of homogeneous heating and simple nucleation of noble metal NPs. A fast NPs generation method (within few seconds) for the synthesis of Au, Ag, palladium and platinum in aqueous medium by MW irradiation method at 50 W is reported by using red grape pomace as a reducing agent.

### Bio-based methods

Bio-based methods are more useful for the production of highly stable, well characterized and safer NPs than the chemical methods, which are usually not environment friendly, less stable and not easy to scale up. There are few examples elucidated and elaborated in further sections of the review. Bacteria and actinomycetes Shivaji et al. developed Ag NPs stable in dark place for 8 months by using cell-free culture supernatants of psychrophilic bacteria Pseudomonas antarctica, Pseudomonas proteolytical, Pseudomonas meridian!`, Arthrobacter kerguelensis, Arthrobacter gangrenes*, Bacillus indicus and Bacillus cecembensis* Simon Ag suggested that particular gene is responsible for Ag resistance in bacteria and these bacteria can replace the use of Ag in case of burn to reduce chances of the Ag toxicity  Silver nano-crystals of different compositions were successfully synthesized by Pseudomonas stutzeri  AG259.

Yeasts and fungi gold nitrate was transformed into Ag oxide, forming well dispersed NPs, by the action *of F. oxysporum* metabolically. The introduction of Ag particles to *F. oxysporum*, brought about the release of nitrate reductase ensuing development of exceedingly stable Ag NPs in solution  Nano-platinum has been incorporated by the culture filtrate *of Alternaria alternates*, and the platinum NPs were characterized by various spectroscopic investigations (particle size 2–30nm, spherical and triangular found the O–H stretching, C–H stretching (proteins and other organic residues), amide I (polypeptides) amide III bands (the random coil of protein)). The synergistic action of selenium NPs and the fungal C. albicans was observed after combination of selenium NPs with chitosan and fungi was used as reductive agent. Extracellular biosynthesis of AgNPs from Ag nitrate solution is reported by the fungus Trichoderma viride*. Fusarium oxysporum* has been used to develop very stable Ag NPs of sizes 5–15nm.

Algae Cyanobacteria and eukaryotic green development genera, for instance, L. majuscule*, S. subsalsa*, R. hieroglyphics, *C. vulgaris, C. prolifera, P. pavonica*, *S. Platensis and S. fluitans* can be used as cost effective materials for bio recovery of metal out of the liquid courses of action Uma Suganya et al. inspected green synthesis of Au metal NPs by using blue green development. The product of AuNPs was a direct result of the reduction of Au3þ particles of chloroauric destructive to Au0 by S. platensis protein.

Plants and phytochemicals Combination of NPs utilizing plants is extremely practical, and in this manner can be utilized as a monetary and important option for the expansive scale generation of NPs I an exploration of different antioxidant constituents of the extracts of blackberry, blueberry, turmeric and pomegranate, the pomegranate was found to have the ability to produce more uniform size and shape NPs of Au and Ag in the range of 20–500nm. These NPs could be used for the management of cancer and the antioxidant therapy F. herba isolate was used to reduce the platinum compound, the closeness of hydrogen and carbonyl in polyphenolic compound mainly goes about as fixing expert for metal particles [35]. Formation of NPs could be completed in salt solution within short duration of time depending on the nature of plant extracts; the main reason being the concentration of the extracts, metal salt, pH and contact. It has been discovered that decrease of AgNO3 to AgNPs by dihydroquercetin,

quercetin and rutin prompted the development of an intensive surface plasmon resonance (SPR) band, which suggests reduction of this constituent [36]. Kou and Varma reported a simple, green and fast (complete within 5min) approach for the construction of Ag NPs by MW irradiation using beet juice as a reducing reagent. The prepared material displayed good photocatalytic activity for the degradation of methyl orange (MO) dye.

Metals synthesized from green synthesis Copper (Cu) and copper oxide (CuO)

Colloidal heat combination process is utilized to get CuO nanomaterials. The incorporated *CuO* was decontaminated and dried to acquire distinctive sizes of the CuO NPs. Manoj et al. reported a method of developing a bio- sensor for the detection of nitrite ions in the medium, this method includes use of CMC as substrate for developing highly stable and sensitive nanocomposite of Cu.

## Zinc oxide (ZnO)

Cassia auriculata blossom extract was utilized for the treatment of fluid arrangement of Zn (NO3)2 to combine stable ZnO NPs with normal size extents 110–280nm. Alijan et al. observed the normal size of ZnS NPs to be 8.35nm while being exceptionally steady and overwhelmingly spherical ZnS NPs were orchestrated utilizing a characteristic sweetener glycoside (250–300 times sweeter than sucrose) in the aqueous rough concentrate of *Stevia rebaudiana* that went about as a great bio-reductant. ZnO and Ag/ZnO NPs obtained through green synthesis are also useful in clinical antimicrobial wound-healing bandages (Khatami *et al.,*2018).

## synthesis of nanoparticles-:

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Cerium oxide ($CeO_2$) Rocca et al. investigated the antioxidant effects of $CeO_2$ NPs as a potential pharmaceutical approach for the treatment of obesity Moreover, besides possessing fast electron transfer kinetics, CNP is an excellent co-immobilization material for a variety of enzymes such as cholesterol oxidase, glucose oxidase and horseradish peroxidase Extract of Gloriosa superba leaf displayed excellent antibacterial properties; $CeO_2$ NPs were of spherical shape with an average size of 5nm Miri and Sarani conducted the cytotoxic investigation of cerium oxide NPs ($CeO_2$-NPs) biosynthesized utilizing the aqueous extracts of ethereal parts of *Prosopis farcta*, and demonstrated that the biosynthesized particles were consistently and roundly formed with a size of around 30nm.

## Cadmium sulphide (CdS)

Cadmium sulphide quantum dots were developed from the plant synthesis of CdS NPs by Biomass of Fusarium oxysporum get dots of sizes ranges between 2–6nm (Ardenas et al.,2017). assisted method was used to produce Cds NPs of Trichoderma harzianum, a common bio fungicide, it produced 3–8nm spherical wurtzite CdSNP after 72h of biomass incubation with cadmium chloride and sodium sulphide (Bhadwal et al,.2014) In a study of Escherichia coli and Klebsiella pneumonia (isolated from the stool of healthy volunteer's samples) it was found they possess the ability to produce CdS NPs. This kind of microorganisms can be used for synthesis of NPs and heavy metal absorption for detoxification of environment

Silver and gold Silver and Au NPs have been broadly considered for use in application in a different scope of fields (e.g., opto electronics, catalysis, sensing, medicine, etc.). Honey can increase the reduction speed as the concentration is increased in the NPs solution, NPs formed with the mediation of honey are having special characteristics such as bio-sensing, anticorrosive, catalytic and antimicrobial activity. Francis et al. prepared Au and Ag NPs using M. glabrata leaf extract from their respective metal salt precursors by MW assistance. They open a new area for water purification because of their tremendous antimicrobial activity inhibiting pathogenic microorganisms like Bacillus pumilus, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger and Penicillium chrysogenum. An economic method was developed to synthesize Ag NPs of smaller then 140nm sizes by using two different microorganisms *Bacillus subtilus* 10833 and *Bacillus amylococus* 1853, problem with this method was lake of reproducibility, time consuming process (48h) and impurity issue at some extent Shen et al. revealed an investigation in which they reported combination of Au NPs from various microorganisms. They compared three different cell free extracts, i bacteria Labrys sp., yeast *Trichosporon montevideense,* and filamentous fungus Aspergillus sp., selected for AuNPs and at the end of experiment they reported the average sizes of the NPs were 18.8, 22.2 and 9.5nm, respectively. They found that the fungus showed.

better results as compared to (Shen *et al.,*2018). described gold NPs (AuNPs) from concentrates of Cucurbitapepo L. takes off. The examination was completed at various plant ages, from one to four months, and the generation of NPs (in term of size, shape and yield) was dependent on the concentration of chlorophyll and carotenoids in the extracts. (Sarani *et al.,* 2018). Green combination of Ag NPs was created, utilizing a low-toxic system of microemulsion and nano emulsion with castor oil as the oily phase, Brij 96V and 1,2-hexanediol as the surfactant and co-surfactant individually. *Geranium (P. hortorum)* leaf aqueous extract was utilized as a reducing specialist. Green synthesis of silver nanoparticles in oil-in-water microemulsion and nano-emulsion using geranium leaf aqueous extract as a reducing agent. Colloids Surfaces (Rodriguez *et al.,*2018).

## **Characterization**

After the synthesis of NPS it is must to ponder the morphology and other conformational subtle elements by utilizing different spectroscopic strategies. The most widely utilized systems are: UV–vis absorption spectroscopy, X-ray diffraction (XRD), Fourier transmission infrared (FTIR) spectroscopy, dynamic light scattering (DLS), energy dispersive X-ray examination (EDAX), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and so on.

## UV–visible spectroscopy

Arrangement of NPs from UV–visible spectroscopy can be studied due of their surface plasmon reverberation assimilation band because of the consolidated wavering of conduction band electrons on the surface of metal NPs in reverberation with light wave*. Subhapriya and Gomathipriya* reported the bio-reduction of Ag nitrate to Ag NPs and estimated occasionally by UV–visible spectroscopy.

## FT-IR-

FT-IR spectroscopy is conducted to discover data about the diverse utilitarian gatherings from the pinnacle positions in the range, FTIR spectroscopy is used to examine the property of functional groups or metabolites present on the surface of NPs, which might be responsible for reduction and stabilization of NPs and information about capping and stabilizing of the NPs (Shankar *et al.,* 2015).

## High resonance SEM

New age of high-resolution SEM (HRSEM) permits a determination superior to anything 1nm, moving towards the determination of TEM. With this procedure, it is conceivable to break down collaborations, for example, the adsorption and take-up of metallic NPs by cells. TEM and SEM examination clarifies the morphology and size of the resultant NPs. TEM results uncover that the Ag NPs are round and monodisperse.

## XRD spectroscopy

X-ray diffractograms of nano-materials give an abundance of data from phase creation to crystallite estimate, from cross section strain to crystallographic introduction, XRD is non-contact and non-destructive, which makes it ideal for in situ studies  Other techniques for characterization of NPs are EDS and DLS, the energy dispersive spectroscopy (EDS) is used to separate the characteristic X-rays of different elements into an energy spectrum, is used for detection of elemental composition of metal NPs. Dynamic light scattering analysis of incident photons is used to determine the surface charge and the hydrodynamic radius of the NPs.

## Applications of green nanotechnology

From recent years, dramatic changes in the interest of researchers developed for the green nanotechnology and number of science publications are expanding ceaselessly. Green NPs have differing impact on the utilization of metallic NPs. They assume an imperative part to expanding the utility of NPs in pharmaceutical field particularly.

## Agricultural engineering

Nanosized ligno-cellulosic materials are gotten from harvests and trees, which had opened-up another market for inventive and worth nano-sized materials and things. Nano-fertilizer, nano-pesticides intertwining nano-herbicides, nano-coating, etc. are the applicability of NPs in this field.

Ag-NPs have been utilized in dental instruments and swathes. Joining of Ag-NPs into orthodontic glue can increase or keep up the shear bond nature of orthodontic cement while expanding its confirmation from microorganisms.

## X-ray imaging

AuNPs have pulled in the primary consideration as ax-ray differentiate specialist since it speaks to a high X-ray retention coefficient, simplicity of engineered control, nontoxicity, surface functionalization for colloidal dependability and focused on conveyance.

## Drug delivery

The broad features of AuNPs, for instance, remarkable optical, physicochemical properties, biocompatibility, viable flexibility, controlled dispersity and nontoxicity make them a compelling nano-carrier in drug delivery systems (DDSs).

# Materials and method

**1. Synthesis of fungus mediated gold nanoparticles.**

Isolated and purified endophytic fungal culture was employed in the synthesis of gold nanoparticles. The isolated *Aspergillus fumigatus.* was maintained on potato dextrose agar (PDA) at 25°C.

**2. UV-Visible Spectroscopy.**

Auric chloride salt (1mM) was incubated with *Aspergillus fumigatus* for 96 hrs and UV- visible spectra was done (O.D.- 0.66) in order to 0 hrs,24 hrs, 48 hrs, 72 hrs and 96 hrs Amalgamation of gold nanoparticles was examined utilizing UV-visible spectroscopy ranges from 220 to 800 nm.

**3. Particle Size Analysis.**

The synthesized nanoparticles were filtered using Whatman syringe filter paper (0.2$\mu$) to eliminate the spores. Filtrate was collected and particle size was detected by Particle Size Analyzer.

## Antimicrobial properties of gold nanoparticles

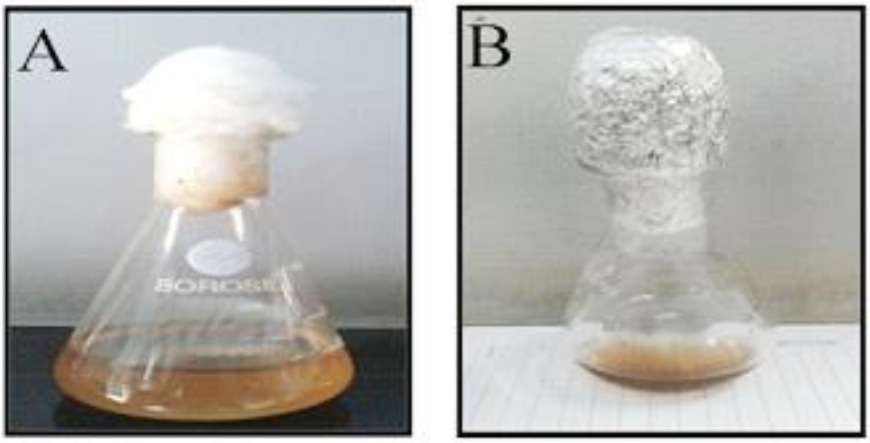
Biosynthesized silver nanoparticles from of the fungal culture were studied for antimicrobial properties against pathogenic bacteria (clinical isolates) using agar disc diffusion assay method (Bauer et al., 1966) separately. The MTCC culture of the test organisms used were aspergillus fumigatus. The microbes were grown in nutrient broth for 12h. Lawns o were prepared on nutrient agar plates using sterile cotton swabs. Agar discs were placed on nutrient agar plates and each disc was loaded with 25µl of gold nano particle solution. The plates containing bacteria and gold nanoparticles discs were incubated at 37oC for 24 to 48 hours in the BOD incubator. The plates were examined for the zone of inhibition after 24 hrs. which appeared as clear are zone around the wells. Inhibition zone diameter was measured in mm by the HI-Media scale.

Antimicrobial properties of synthesized SNP

In order to assess the antimicrobial effect of synthesized gold nanoparticles, one standard (*Aspergillus fumigatus and Aspergillus parasiticus* strains were applied. The isolates was kept at -80° C as 20% glycerol stocks and were sub-cultured, as required, on SDA plates at 30°C. Microdilution antifungal susceptibility testing of the Aspergillus Fumigatus isolate was performed by the broth microdilution method described in CLSI (Clinical and Laboratory Standards Institute) document M27-A3 (32) for SNPs. Fluconazole was used as a reference anti agent. RPMI 1640 medium with L-glutamine and phenol red without bicarbonate was used. The medium was buffered to pH 7.0 with 0.165 mol/L MOPS (3-(N-morpholino) propane sulfonic acid). Fluconazole was dissolved in sterile diluted water to 64µg/L, and then diluted to the final concentration of 0.0125-16 µg/L with the medium according to the standard in the CLSI reference method. After growing on SDA (Merk, Germany) at 35°C for 24 h, the yeasts were re-suspended in culture medium to prepare a working solution at a concentration of 2.5×103 cells/mL. The prepared yeast solution was exposed to diluted fluconazole (0.0125-64 µg/L) and finally incubated at 35°C for 48 h. All tests were carried out in duplicate. The interpretive criteria for susceptibility to fluconazole were published by the CLSI (32) and are as follows: (i) susceptible ≤8 µg/mL; susceptible dose-dependent (S-DD) 16 to 32µg/mL; and resistant ≥64 µg/mL for silver nanoparticles, a solution of 16µg/mL was prepared and diluted to the final concentration of 0.0125–16 µg/L with the medium. The same inoculum size was applied for SNPs susceptibility test and finally, the 96-well plate was incubated at 35° C for 48 h

## Results and Discussion.

The mycelium indicates yellow shading before response with the auric particle, which changed to a purple shading after completing of the response (Figure1). Appearance of a burgundy red shading in arrangement incorporate the biomass was clear indication of the improvement of nanoparticles in the response blend and was because of the excitation of surface plasma on vibrations in the nanoparticles (Ahmad et al., 2003).




Conical flasks containing fungal biomass in the aqueous solution of 1mM Gold salt (A) at 0 hr and (B) at 96 hrs. of reaction.

The synthesis of nanoparticles was examined via spectral scan by UV-visible spectroscopy in the region between 220–800 nm (Henglein, 1993). A broad peak located between 350 and 500 nm was found to increase with time representing the gold nanoparticles synthesis. The peak was produced in 120 min of response test demonstrating that the synthesis of gold nanoparticles begins rapidly. The peak reached to near saturation at 360 min reaction time shown in Fig.

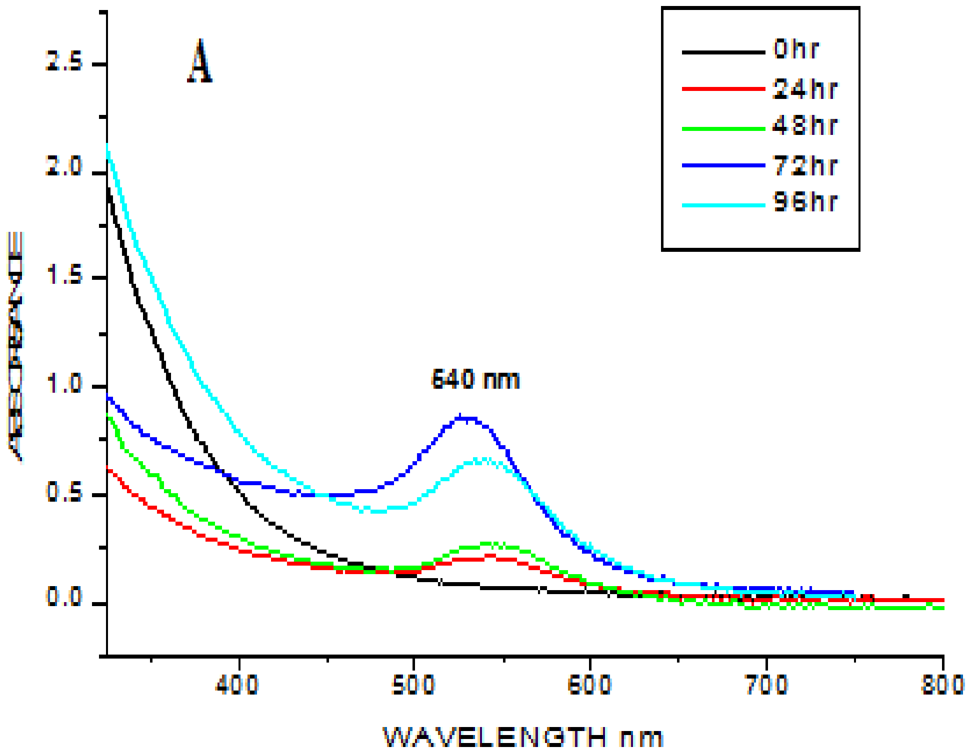



**Fig.2:** Curves correspond to UV- Vis spectra from the GNP-SPI phase after 0, 24, 48, 72 and 96 hrs of exposure to the biomass respectively.

Biosynthesized gold nanoparticles were found to be of 49 nm size using particle size analyze (Figure 3). The nanoparticles were not in direct contact within the aggregates, indicating stabilization of the nanoparticles by capping agents. The solution of gold nanoparticles synthesized by the reaction of $Au^{3+}$ ions with fungal biomass was exceptionally stable.

If the aim is to employ nanoparticles in biomedicine as therapeutical gents in cells .it is essential to choose the targeting component such as a monoclonal antibody (m Ab) (Cao-Milan and Liz-Marzán, 2014). GNPs have also been used as successful agents for therapeutic applications (Huang, 2008).

A physical relation between fungal mycelium and nanoparticles depends on three phenomena: (a) ionic attraction nanoparticles and hydrophobic attraction between the extra cellular and the gold surface; (c) dative binding between the gold conducting electrons and amino acid sulfur atoms of the extra or intra-cellular (Ljungblad, 2009).

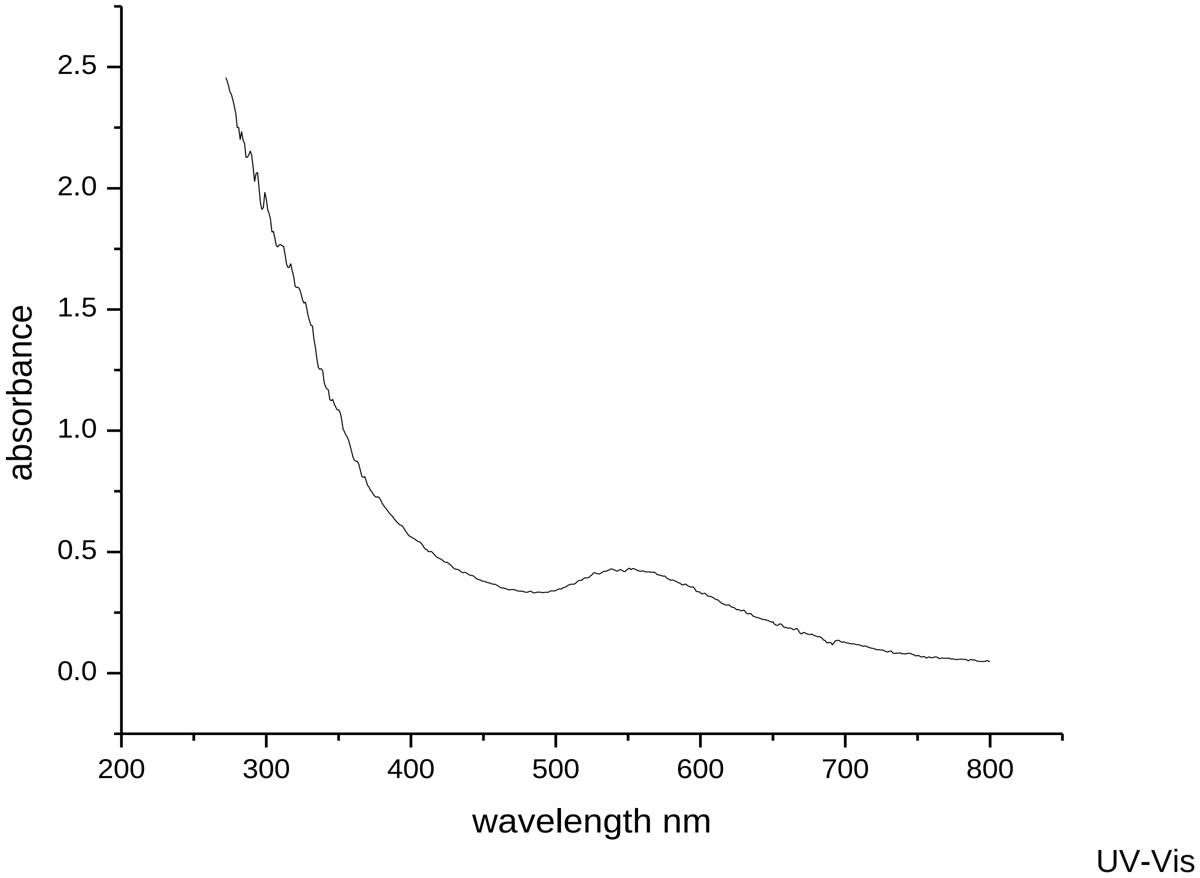



**Fig:2** Spectroscopy of synthesized GNPs

Zeta potential is a physical property which is exhibited by any particle in suspension. It can be utilized to streamline the plans of suspensions and emulsions. It is additionally a guide in foreseeing long term stability. The sample was diluted in phosphate buffer and afterward the molecule appropriation in the fluid was examined in a computer controlled molecule size analyzer (ZETA sizer man settings nano, Malvern instruments Nano Zs) shown in fig.5.4.

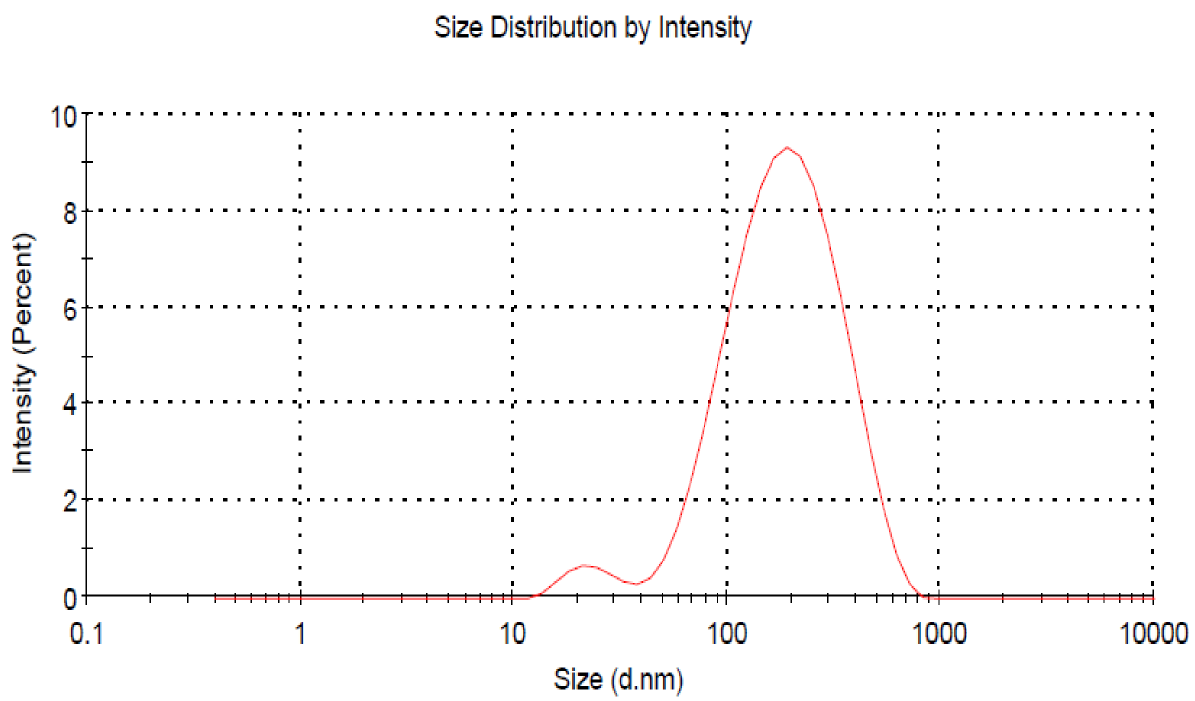



**Fig. 3** Zeta potential of synthesized GNP

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was utilized to think about the normal molecule size of GNP nanoparticles. The prepared sample was scattered in deionized water took after by ultra-sonication.

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