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A mini review on fungal based synthesis of silver nanoparticles and their antimicrobial activity

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Abstract : new green chemistry approach for the synthesis of silver nanoparticles based on myconanotechnology has been identified as one of the novel field of study in nanotechnology. Researchers have re-explored the fungi including yeast and filamentous fungi as eco-friendly, cost effective source for the synthesis of nanoparticles. The advantage and most notable benefits of applying fungi in nanoscience is resistance to many harsh conditions and secretion of extracellular reductive protein makes the downstream process easier. This review focuses on general introduction of fungi, synthesis of silver nanoparticles its physico-chemical characterization and its antimicrobial activity.
Key words: Silver nanoparticles, Fungi, Biosynthesis, Antimicrobial activity, Eco-friendly.

Introduction

Nanotechnology

Nanotechnology provide a platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostic, biomarkers, cell labelling, contrast agents for biological imaging, antimicrobial agents, drug delivery system and nano drugs for treatment of various disease[1]. Nanotechnology is a multidisciplinary field that covers wide range of chemical, physical, biological, electrical and electronics engineering. Synthesis of nanoparticles can be carried out by two different approaches, either from the bottom up or top down[2]. The top down approach involves a physical or chemical process of breaking down larger particles of food matter into smaller particles of nanometers in dimensions[3].

Nanotechnology has maximized the applications by minimizing the size of particles. The unique size dependent properties make these materials superior and essential in many areas of human activity. Nanotechnology has numerous applications in various fields like medicine, food industries, agricultural fields etc[4]. Many environmental and technological challenges are being solved by nanoparticles of desired size and composition[5]. It is an important field of modern research dealing, synthesis and manipulation of particles structure ranging from approximately 1-100nm. Tremendous growth in this emerging technology including the synthesis of nanoscale materials and utilization of their exotic physic-chemical for optoelectronic properties inspired researcher to focus on this field. Nanotechnology provides a good platform for having promising applications in diagnostics, antimicrobial agents and drug delivery system[6]. In recent years there is an enormous growth in the interdisciplinary world of nanotechnology across the globe and emergence of its potential applications remains as a big revolution in the industry. The aim of developing nonmaterial for diagnosis, treatment prevention of various diseases and overall improvement of health for the beneficial of mankind[7].

Silver Nanoparticles

Nanoparticles defined as a dispersion of solid particles with size in the range of 1-100 nm. Nanoparticles are the building block of nanotechnology which plays a major role in their applications. The applications of biosynthesis of nonmaterials provide solution to technology challenge in the area of solar energy conservations, catalysis, antimicrobial activity and water energy treatment[8]. Silver has been used for medicinal purpose in the field of health care from ancient period. Recently Silver nanoparticles (AgNPs) have been found to be a potent antimicrobial agent, thus replacing elemental silver in all aspects.

Hence the development of experimental procedure for the synthesis of silver nanoparticles of various sizes, shapes, chemical compositions, and controlled polydispersity are significant[9]. The nanoparticles created by many microorganisms can provide inorganic materials either intracellular or extracellular. For example, Extracellular biosynthesis of silver nanoparticles using fungi *T.Reesi* shows individual silver nanoparticles size range from 5-50 nm[10]. The size and shape of the intracellular synthesized nanoparticles are spherical in shape and elongated particles. *F.oxysporium* shows size range from 25 nm and some particles are agglomerated [11].

Synthesis of Silver Nanoparticles using Fungi

Fungi are easy to culture on large scale by solid substrate fermentation and thus large scale silver nanoparticles are formed. Fungi have tendency to form product intracellular as well as extracellular and it has high cell wall binding capacity and metal intake capacity. Synthesis of silver nanoparticles has been investigated utilizing many ubiquitous fungal species including *Tricoderma*[12],*Fusarium*[13], *Penicillium*[14], *Pleurotus*[15], and *Aspergillus*[16]. Extracellular synthesis has been demonstrated by *Trichoderma viridae*[17],*T.reesi*[10],*Trichoderma inhamatum*[12], *Fusarium oxysporum*[18], *F.semitectum*[19], *F.solani*[20], *Aspergillus niger, A.flavus*[2], *A.fumigatus*[22], *A.clavatus*[23], *Pleurotus ostreatus, Cladosporium cladosporioides*[12], *Penicillum brevicompactum*[24], *P.fellutanum*[25], an endophytic *Rhizoctonia sp*[26], *Epicoccum nigrum*[27], *chrysosporium tropicum*[28], while intracellular synthesis was shown to occur in a *verticillium* species and *Neurospora crassa*[29].

The synthesis of silver nanoparticles by fungi is following several ways: Surface of the fungal cells was trapping the Ag^+ ions and the subsequent reduction of the silver ions by the enzymes present in the fungal cell wall[30]. Naphthoquinones and anthraquinones are extracellular enzyme presented into the fungal cell wall said to facilitate reduction. Considering the example of *F.oxysporium* it is believed that the Nicotinamide adenine dinucleotide phosphate (NADPH) dependent nitrate reductase and a shuttle quinine extracellular process are responsible for the nanoparticles formation[31]. *Clasdosporium cladosporioides* is used to synthesize silver nanoparticles. In thismethod the release of proteins, organic acids and polysaccharides are responsible for the formation of spherical crystalline silver nanoparticles[32].The culture filtrate of *Penicillium fellutanum* was incubated with silver ions and maintained under dark conditions, spherical nanoparticles are formed. They also changed the factors such as pH, incubation time, temperature and concentration of silver nitrate to achieve the maximum nanoparticles production [25]. When compared to bacterial synthesis silver nanoparticles, fungal synthesis of nanoparticles producing large amount of nanoparticles. Fungal organisms secrete more amounts of proteins which help in the higher productivity of nanoparticles[33]. Scheme of the synthesis of silver nanoparticles, characterization and antimicrobial activity is given in Figure.1.

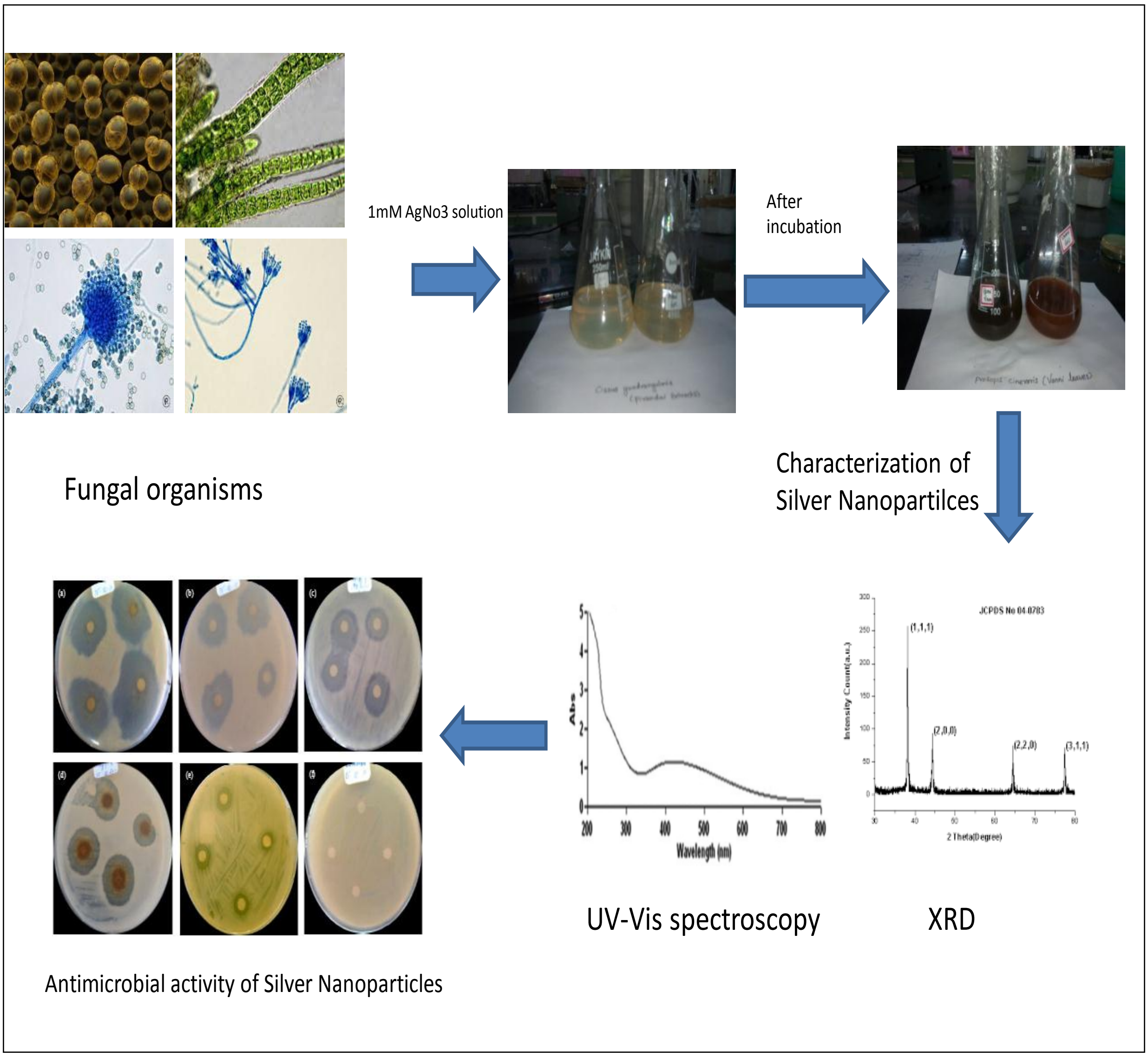


Figure No.1: Fungal synthesis of Silver Nanoparticles

Synthesis of Silver Nanoparticles using other Sources

Biosynthesis of nanoparticles using plant extract involves very rapid reduction of metallic materials. Nowadays, use of plants for the formation of silver nanoparticles has gained the attention of researches because of its rapid, economic, eco-friendly in nature. It also provides single step technique for the synthesis process. In plant based nanoparticles flavonone and terpenoid component are present in the stabilized form in comparison of fungal biomass[34]. In plants, the polyol components are mainly responsible for reduction of silver ions and stabilization of nanoparticles. The leaf extracts of pine plant contains bio molecules such as alkaloids, proteins/enzymes, amino acid, alcoholic compounds which are responsible for formation of silver nanoparticles in the solution [35].Bacterial synthesis of silver nanoparticles has advantages compare to the other biological sources because of short period of cultivation and easy handling. These bacterial generated nanoparticles possess well defined shapes such as pyramidal and hexagonal silver nanoparticles up to 200 nm in size. Synthesis of nanoparticles in both intracellular and extracellular are observed from variety of bacteria.

Silver nanoparticles synthesis using microbes is due to their resistance mechanisms. The resistance caused by the bacterial cell is responsible for nanoparticles production. Also, temperature and pH plays an important role in the production of nanoparticles. Room temperature (28^{o}C) synthesized silver nanoparticles the size of the nanoparticles is 50 nm.Whereas, at higher temperature(60^{o}C) the size of the nanoparticles reduces to

15 nm. Under alkaline conditions, silver nanoparticles produced are more when compared to acidic conditions. The silver nanoparticles synthesis from *Pseudomonas stutzeri* AG259 bacterial strain isolated from silver mines is reported [36]. Moreover silver nanoparticles have been synthesized by many bacterial species such as *B.subtilis*37, *Pesudomonas stutzeri*38, *Klebsiella pneumonia*[39], *E.coli* [40],*Enterobacter cloacae*[41], *Aeromonas sp*[42], *Corynebacterium sp*[43],*Lactobacillus sp*[44].

Physico - Chemical Characterization of Silver Nanoparticles

Nanoparticles are characterized byseveral techniques such as Ultraviolet visible Spectroscopy (UV-Vis), Scanning electron microscopy (SEM), Powder X -ray diffraction (XRD).

Table No.1: UV analysis of silver nanoparticles synthesized using various fungal organisms[48,49,50,21,15,51,47,52,53,54,55,56,57]

Fungi	Λmax
F.oxysporium	430nm
As.niger	420nm
Collectricum sp	420nm
As.fumigatus	425 nm
T.Reesei	414-420 nm
P.ostreatus	440nm
Penicillium sp	420-450 nm
Saccharomyces cerevisiae	430nm
Trichoderma species	420nm
Guignardia mangiferae	417nm
Humicola	415nm
Penicillium atramentosum	420nm
pestalotia sp	415nm
Rhizopus stolanifer	422 nm

UV-visible Spectroscopy

The rapid colour changes from colourless to yellowish dark brown colour indicate the formation of silver nanoparticles. In this reaction Ag^+ reduced to Ag^o with aqueous solution the formation of silver nanoparticles (AgNPs) is analyzed. Silver nanoparticles synthesized using various fungal organisms with their maximum wavelength in UV analysis are given in Table 1.The typical peak at 450 nm corresponds to the absorbance wavelength of AgNPs. It is also reported that the silver nanoparticles exhibits a range of wavelength from 390 to 420 nm due to the Mie scattering [45]. Silver nanoparticles are synthesized in different pH from 5 to 13 using *Penicillium sp*[46]. Saeed Moharrer et al., reported thesynthesis of silver nanoparticles using *Aspergillus flavus* which shows the absorption maximum at 425 nm 21. Whereas *As.clavatus* synthesized AgNPs exhibits the wavelength maximum at 420 nm 47. The UV –Visible absorption spectrum of fungal synthesis silver nanoparticles synthesized using is given Figure. 2.

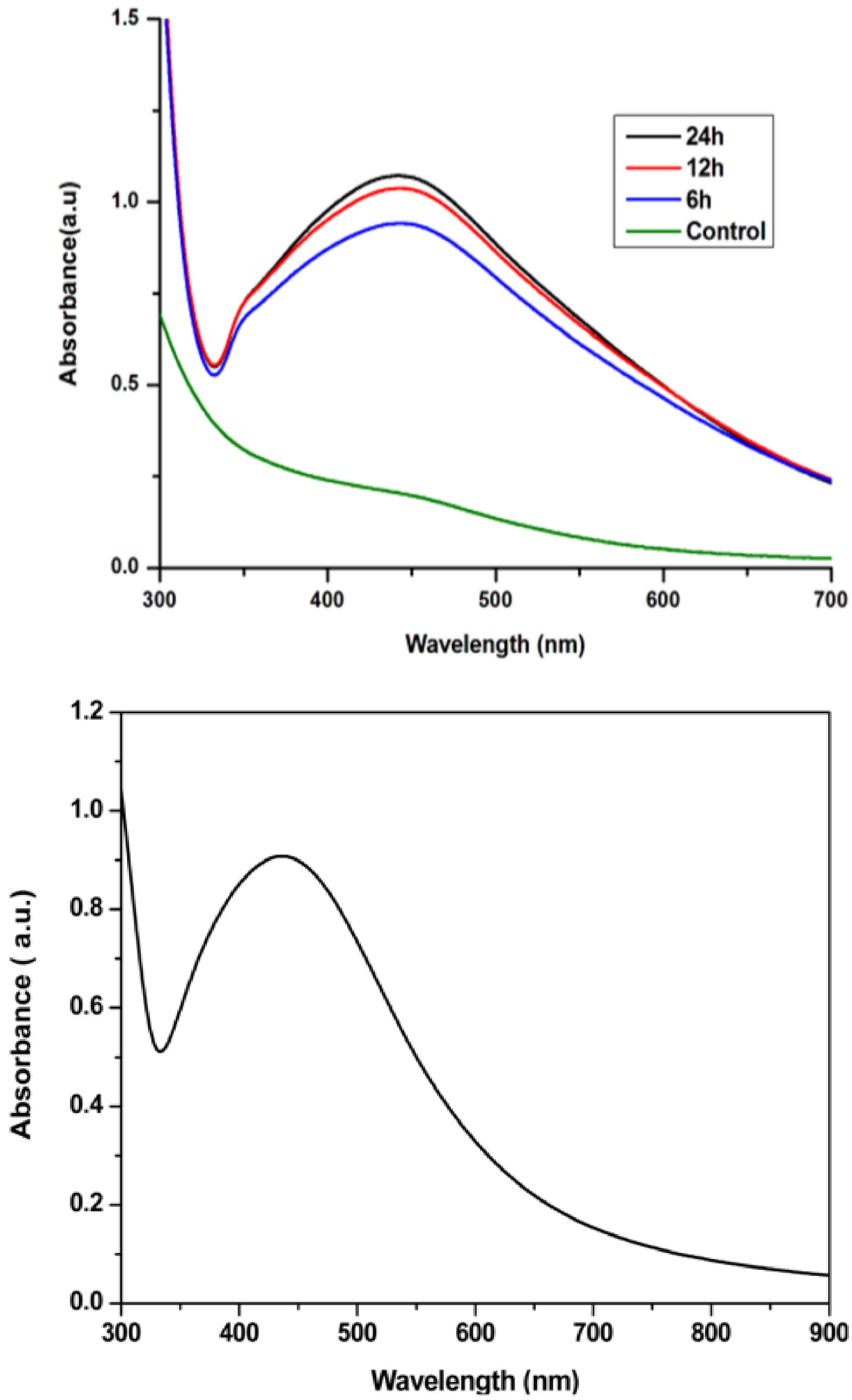


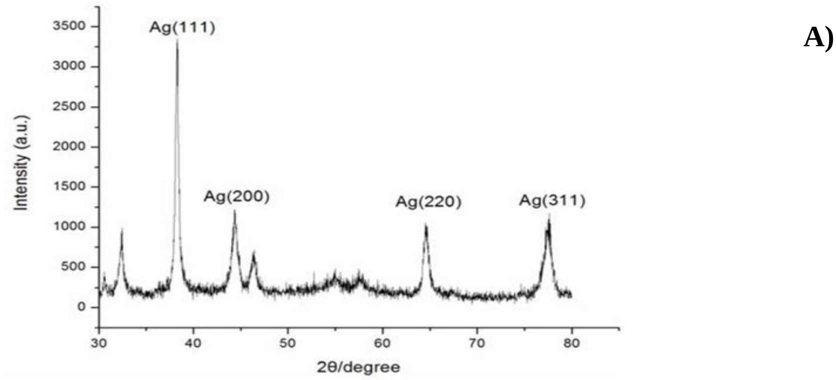
Figure No. 2: UV analysis of AgNPs using A) As.terreus [58] B) Penicillium sp [59]

Powder X-ray Diffraction

The crystalline nature of the synthesized nanoparticles depicted with Bragg's peak 38.2°, 44.4°, 64.5°, 77.4°. The X-ray diffraction of silver nanoparticles using *As.oryzae* and *As.terreus* are given in Figure.3. XRD analysis showed three clear diffraction peaks corresponding to the (111), (200), (220) planes confirm the formation of AgNPs.Liangwei Du et al synthesized silver nanoparticles using *penicillium oxalicum* at two different pH- 8 and pH-12[61]. Irrespective of the values of pH the AgNPs showed four characteristics diffraction peaks at 38.2°, 44.4°, 64.7°, 77.7°. These are corresponding at (111), (200), (220) and (311) Bragg's reflections respectively. The intensity of the (111) diffraction was much stronger than (200). Scherrer equation is used to calculate average crystalline size from the full width of half maximum of the (111).AgNps synthesized from *As.terreus* was examined by the XRD pattern showed 2 theta values at 32.3°, 45.1°, 75.9° assigned to the planes of (111), (200), (311) corresponds to faced centered cubic structure of AgNPs.

Scanning Electron Microscope (SEM)

The size and shape of the silver nanoparticles is analyzed using scanning electron microscopy. *F.oxysporum* silver nanoparticles were almost spherical in shape of size 25-50 nm, these attached to the surface of fungal cell [62]. Binupriya et al.,[17]synthesized, *R.stolanifer* based silver nanoparticles having a size of 25-30 nm which is Quasi-spherical in shape. Spherically poly dispersed (10-40 nm) silver nanoparticles were synthesized using *T.viridae* by Fayaz et al., 2009[63]. Kathiresan et al., [25] used the pathogenic fungi *P.fellutanum* to synthesize Hexagonal or spherical nanoparticles of size vary from 10-25 nm.



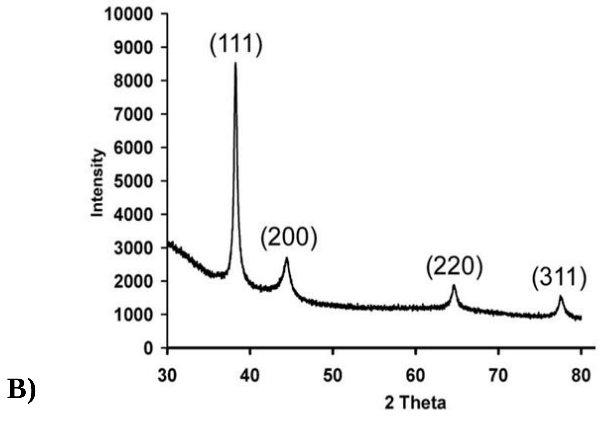


Figure No.3: XRD pattern of AgNPs using A) *As.oryzae*[60], B) *As.terreus*[58].

Table No. 2:Size and shape of the AgNPs[64,65,20,66,21,67,68,69,70,24].

As.fumigatus	5-25 nm	Spherical
P.chrysosporium	5-200 nm	Pyrimidal
F.solani	16.23 nm	Spherical
As.flavus	7 nm	Spherical
As.niger	10-60nm	Spherical
F.Semitectum	15 nm	Spherical
V.volvacea	25-75 nm	Sperical and Hexagonal
Agaricus bisporus	5-50 nm	Spherical
As.terreus	100 nm	Spherical

Antimicrobial Activity of Silver Nanoparticles

The antimicrobial activity of *Penicillium sp* synthesized AgNPs against bacterial pathogens *Bacillus cereus* (13mm) and *E.coli* (12mm) are reported by Shanaz Majeed et al.,[71]. This activity was carried out by Disc diffusion method and each petridish has been impregnated with 25 µl AgNPs solution. *Aspergillus niger* AgNPs was synthesized as extracellular nanoparticles at room temperature. The spherical shape nanoparticles ranged in size from 10-50 nm and showed excellent antimicrobial activity against *S.aureus* and *E.coli*. These *As.niger* organisms are highly potential for the green, sustainable production ofAgNPs[72]. Synthesized using *Aspergillus terreus* showed inhibition against dermatophytic fungi *T.rubrum*. *E.floccosoum* source of silver nanoparticles suggests the future use as antidermatophytic drugs/agents 24. Synthesis of *Pl.ostreatus* AgNPs also showed activity against *Vibriocholera* (28mm) and *K.pneumoniae* (8mm). *Pl.ostreatus* synthesized silver nanoparticles showed strongest antimicrobial effect on *Vibrio cholera* at 28 mm. AgNPs synthesized from *Pl.ostreatus* species seems to be promising and effective antimicrobial agent against the water borne pathogenic bacteria [15].

Sujatha et al., [70]reported the mushroom extracts (*A.bisporus*, *C.indica*, *P.florida)* synthesized AgNPs with good antimicrobial activity against both Gram positive and Gram negative microorganisms. AgNPs synthesized using *A.bisporus*shows interaction with bacterial cells and strong inhibition against *E.coil* (12mm), *E.aerogenes* (18mm), *S.aureus*(12mm)[70]. The antimicrobial activity of *S.torvum* mediated AgNPs are reported against *P.aeroginosa* (17.9 mm), *S.aureus* (16.9 mm) and silk worm [73]. Antimicrobial activity of AgNPs synthesized using fungi against the various pathogenic bacteria are given inTable 3.Fungal synthesized AgNPs were attached to the surface of the microbial cell membrane and disturb the power function of permeability and respiration. The binding of the AgNPs to the bacteria depends on the surface available for interaction. Smaller AgNPs having the larger surface area when compare to larger AgNPs. Smaller particles will be give more bactericidal effect and interact with the microbial cell wall. Silver ions have been shown to react with the thiol group in enzymes and inactivate them, leading to cell death. These ions can inhibit oxidative enzymes such as yeast alcohol dehydrogenase. Silver ions have also been shown to interact with DNA to enhance pyrimidine dimerization by the photodynamic reaction and possibly prevent DNA replication wall [74].

Conclusion

Plant synthesis silver nanoparticles are attracted the attention of researchers because of their unique properties and applicable areas such as medicine, biotechnology, catalysis, electronics, optics, and waste water treatment. Moreover, silver nanoparticles were significantly acted against microbial pathogens and widely used as antibacterial and antifungal agent. The flexibility of silver nanoparticles synthetic methods and facile incorporation of silver nanoparticles into different media have interested researchers to investigate the antimicrobial, antiviral and anti-inflammatory activities of these nanoparticles. Fungi present a suitable option for large scale green nanoparticles production. They are easy to handle during downstream processing and they secrete large amounts of enzymes needed in the reduction. Silver nanoparticles also present filamentous tolerance towards metals, high binding capacity, and intracellular uptake.

TableNo. 3: Antimicrobial activities of AgNPs[47,24,15,71,72,70,76]

Organisms	Size	Type	Antimicrobial Activity	Zone of inhibition
S.cerevisiae	60-80 nm	Extracellular	*P.aeroginosa* *K.pneumoniae*	22 mm 20 mm
As.terreus	100 nm	Extracellular	*T.rubrum* *E.floccosum*	13 mm 15 mm
Pl. ostreatus	50 nm	Extracellular	*K.Pneumonia* *Vibrio cholera*	08 mm 28 mm
Penicilliumsp	20-45 nm	Intracellular	*Bacillus cereus* *E.coli*	13 mm 12 mm
As.niger	10-50 nm	Extracellular	*S.aureus* *E.coli*	14 nm 12.5 nm
A.bisporus	5-50 nm	Extracellular	*S.aureus* *E.aerogenes*	12 mm 12 mm
C. indica	5-50 nm	Extracellular	*E.coli* *E.aerogenes*	12 mm 11 mm
Pl. Florida	5-50 nm	Extracellular	*E.aerogenes* *E.coli*	18 mm 16 mm
S. torvum	14 nm	Intracellular	*S.aureus* *P.aeroginosa*	17.9 mm 16.9 mm
T. viridae	100 nm	Extracellular	*Shigellaboydii* *S. typhimurium*	28 mm 25 mm
F.oxysporium	13 nm	Intracellular	*E.coli* *S.aureus*	20 mm 16 mm

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