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# Fabrication of Metal Nanoparticles from Fungi and Metal Salts: Scope and Application

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## Abstract

Fungi secrete enzymes and proteins as reducing agents which can be used for the synthesis of metal nanoparticles from metal salts. Large-scale production of nanoparticles from diverse fungal strains has great potential since they can be grown even *in vitro*. In recent years, various approaches have been made to maximize the yield of nanoparticles of varying shape, size, and stability. They have been characterized by thermogravimetric analysis, X-ray diffractometry, SEM/TEM, zeta potential measurements, UV-vis, and Fourier transform infrared (FTIR) spectroscopy. In this review, we focus on the biogenic synthesis of metal nanoparticles by fungi to explore the chemistry of their formation extracellularly and intracellularly. Emphasis has been given to the potential of metal nanoparticles as an antimicrobial agent to inhibit the growth of pathogenic fungi, and on other potential applications.

**Keywords:** Green synthesis, Metal nanoparticles, Antimicrobial, Fungi, Plant

## Review

### Introduction

Of all the processes developed so far, the fabrication of metal nanoparticles by the biogenic methods employing plant extract are more popular, innocuous, inexpensive, and environmentally friendly as they do not leave hazardous residues to pollute the atmosphere [1–6]. Chemical methods for the synthesis of nanoparticles are common, but their use is limited. The biogenic synthesis is, therefore, the best choice where inherently benign organic molecules do not pose a threat to human health and atmosphere. Microbes have a promising role in the fabrication of nanoparticles due to their natural mechanism for detoxification of metal ions through reduction that can be achieved extra- or intracellularly by bioaccumulation, precipitation, biomineralization, and biosorption [4, 7–12].

Use of microorganisms in the green synthesis of metal nanoparticles with special reference to the precious metals using fungi has been done [10, 13–17]. Since fungi contain enzymes and proteins as reducing agents,

they can be invariably used for the synthesis of metal nanoparticles from their salts. Since some fungi are pathogenic, one has to be cautious while working with them during experiment. Fungus biomass normally grows faster than those of bacteria [18] under the same conditions. Although synthesis of metal nanoparticles by bacteria is prevalent, their synthesis by fungi is more advantageous [19] because their mycelia offer a large surface area for interaction. Also, the fungi secrete fairly large amount of protein than bacteria; therefore, the conversion of metal salts to metal nanoparticles is very fast.

Engineered metal nanoparticles of varying size and shape from the diverse fungal species and yeast are listed in Table 1. Extracellular synthesis of nanoparticles involves the trapping of the metal ions on the surface of the cells and reducing them in the presence of enzymes, while intracellular synthesis occurs into the fungal cell in the presence of enzymes. Fungi secrete extracellular proteins which have been used to remove metal ions as nanoparticles. In a broad sense, the metal nanoparticles can be extensively used in different areas of agriculture and technology [2, 5, 6, 20, 21]. Many metal nanoparticles are antibacterial and find extensive uses in medicine [6, 22–24]. The

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**Table 1** Engineered metal nanoparticles of varying size and shape fabricated from fungal and yeast species

Fungi and Yeast	Nanoparticles	Size (nm)	Shape	Location	References
<i>Alternaria alternate</i>	Au	12 ± 5	Spherical, triangular, hexagonal	Extracellular	[25]
<i>Aspergillus clavatus</i>	Au	24.4 ± 11	Triangular, spherical and hexagonal	Extracellular	[26]
<i>A. flavus</i>	Ag	8.92	Spherical	Cell wall	[27]
<i>A. fumigatus</i>	ZnO	1.2–6.8	Spherical and hexagonal	Extracellular	[28]
	Ag	–	–	Extracellular	[29]
<i>A. niger</i>	Au	12.8 ± 5.6	Spherical, elliptical	–	[30]
	Au	10–20	Polydispersed	Extracellular	[31]
<i>A. oryzae</i> TFR9	FeCl <sub>3</sub>	10–24.6	Spherical	–	[32]
<i>A. oryzae</i> var. <i>viridis</i>	Au	10–60	Various shapes (cell-free filtrate), mostly spherical (biomass)	Mycelial surface	[33]
<i>A. sydowii</i>	Au	8.7–15.6	Spherical	Extracellular	[34]
<i>A. terreus</i>	Ag	1–20	Spherical	Extracellular	[35]
<i>A. tubingensis</i>	Ca <sub>3</sub> P <sub>2</sub> O <sub>8</sub>	28.2	Spherical	Extracellular	[36]
<i>Aureobasidium pullulans</i>	Au	29 ± 6	Spherical	Intracellular	[37]
<i>Candida albicans</i>	Au	5	Monodispersed spherical	Cell-free extract	[38]
	Au	20–40	Spherical	–	[39]
		60–80	Non spherical		
<i>C. glabrata</i>	CdS	20 Å, 29 Å	Hexamer	Intra- and extracellular	[40]
	CdS	–	–	Intracellular	[41]
<i>Cladosporium cladosporioides</i>	Ag	10–100	Spherical	–	[42]
<i>Colletotrichum</i> sp.	Au	8–40	Spherical	Mycelial surface	[43]
<i>Coriolus versicolor</i>	Au	20–100, 100–300	Spherical and ellipsoidal	Intra- and extracellular	[44]
	Ag	25–75, 444–491	Spherical	Intra- and extracellular	[45]
<i>Cylindrocladium floridanum</i>	Au	19.05	Spherical	Extracellular	[46]
	Au	5–35	Spherical	Outer surface of the cell wall	[47]
<i>Epicoccum nigrum</i>	Au	5–50	–	Intra- and extracellular	[48]
<i>Fusarium oxysporum</i>	Pt	70–180	Rectangular, triangular, spherical and aggregates	–	[49]
	CdS	–	–	Extracellular	[50]
	Ag	–	–	Extracellular	[51]
	Ag	20–50	Spherical	Extracellular	[14]
	Au	2–50	–	–	[52, 53]
	Au	8–40	Spherical, triangular	Extracellular	[54]
	PbCO <sub>3</sub> , CdCO <sub>3</sub>	120–200	Spherical	Extracellular	[55]
	SrCO <sub>3</sub>	10–50	Needlelike	Extracellular Extracellular	[56]
	CdSe	9–15	Spherical	Extracellular	[57]
	CdS	5–20	Spherical	Extracellular	[58]
	TiO <sub>2</sub>	6–13	Spherical	Extracellular	[59]
	BaTiO <sub>3</sub>	4–5	Spherical	Extracellular	[60]
	ZrO <sub>2</sub>	3–11	Spherical		[61]
<i>F. semitectum</i>	Au	10–80	Spherical	Extracellular	[62]
<i>Hansenula anomala</i>	Au	14	–	–	[63]
<i>Helminthosporium solani</i>	Au	2–70	Spheres, rods, triangles, pentagons, pyramids, stars	Extracellular	[64]

**Table 1** Engineered metal nanoparticles of varying size and shape fabricated from fungal and yeast species (Continued)

<i>Hormoconis resinae</i>	Au	3–20	Spherical	Extracellular	[65]
<i>Macrophomina phaseolina</i>	Ag	5–40	Spherical	Cell-free filtrate	[144]
<i>Neurospora crassa</i>	Au	32 (3–100)	Spherical	Intracellular	[66]
<i>Pediococcus pentosaceus</i>	Ag	–	–	Extracellular	[67]
	Au	–	–	Intracellular	[68]
<i>Penicillium brevicompactum</i>	Au	10–60	Spherical, triangular and hexagonal	Extracellular	[69]
<i>P. fellutanum</i>	Ag	5–25	Spherical	Extracellular	[70]
<i>P. nagiovense</i> AJ12	Ag	25 ± 2.8	Spherical	Cell-free filtrate	[15]
<i>P. rugulosum</i>	Au	20–80	Spherical, triangular, exagonal	–	[71]
		20–40	Spherical		
<i>Penicillium</i> sp. 1–208	Au	30–50	Spherical	Cell filtrate	[72]
<i>Phanerochaete chrysosporium</i>	Au	10–100	Spherical	Extracellular	[73]
<i>Phoma glomerata</i>	Ag	60–80	Spherical	–	[74]
<i>Pichia jadinii</i>	Au	<100	Spherical	Cytoplasm	[7]
<i>Pleurotus sajor caju</i>	Ag	30.5	Spherical	Extracellular	[75]
<i>Rhizopus oryzae</i>	Au	16–25	Spherical	Cell-free filtrate	[76]
<i>Saccharomyces cerevisiae</i>	Au	15–2030	Spherical	Cell wall Cytoplasm	[77]
<i>Schizosaccharomyces pombe</i>	CdS	18 Å, 29 Å	–	Intra- and extracellular	[40]
<i>S. pombe</i>	CdS	1–1.5	Hexagonal	Intracellular	[78]
<i>S. pombe</i>	CdS			Intracellular	[79]
<i>Sclerotium rolfsii</i>	Au	25.2 ± 6.8	Spherical		[80]
<i>Trichoderma asperellum</i>	Ag	13–18	Nanocrystalline	Extracellular	[81]
<i>T. koningii</i>	Au	30–40	Small spheres to polygons	–	[82]
	Au	10–14	Spheres	Cell-free filtrate	[83]
<i>T. reesei</i>	Ag	5–50	–	Extracellular	[84]
<i>T. viride</i>	Ag	5–40	Spherical	Extracellular	[85]
<i>Verticillium</i> sp.	Au	–	–	Intracellular	[86]
<i>Verticillium</i> sp.	Au	20 ± 8	Spherical	Cell wall and cytoplasmic membrane	[87]
<i>V. volvacea</i>	Au	20–150	Triangular, spherical, hexagonal	–	[88]
<i>Yarrowia lipolytica</i> NCIM 3589	Au	15	Hexagonal, triangular	Associated with cell wall	[89]
<i>Y. lipolytica</i> NCIM 3589	Au		Various shape depending on Au <sup>3+</sup> concentration	Intracellular	[90]

antibacterial efficiency is enhanced manifold when a nanoparticle of one metal is coupled with another such as those of copper and silver. Although in recent times several organisms have been investigated for the fabrication of nanoparticles, its mechanism is still not well understood. This review, therefore, focuses on the biogenic synthesis of metal nanoparticles by fungi and attempts to explore the chemistry of their formation extracellularly and intracellularly.

### Synthesis, Mechanism, and Characterization of Metal Nanoparticles

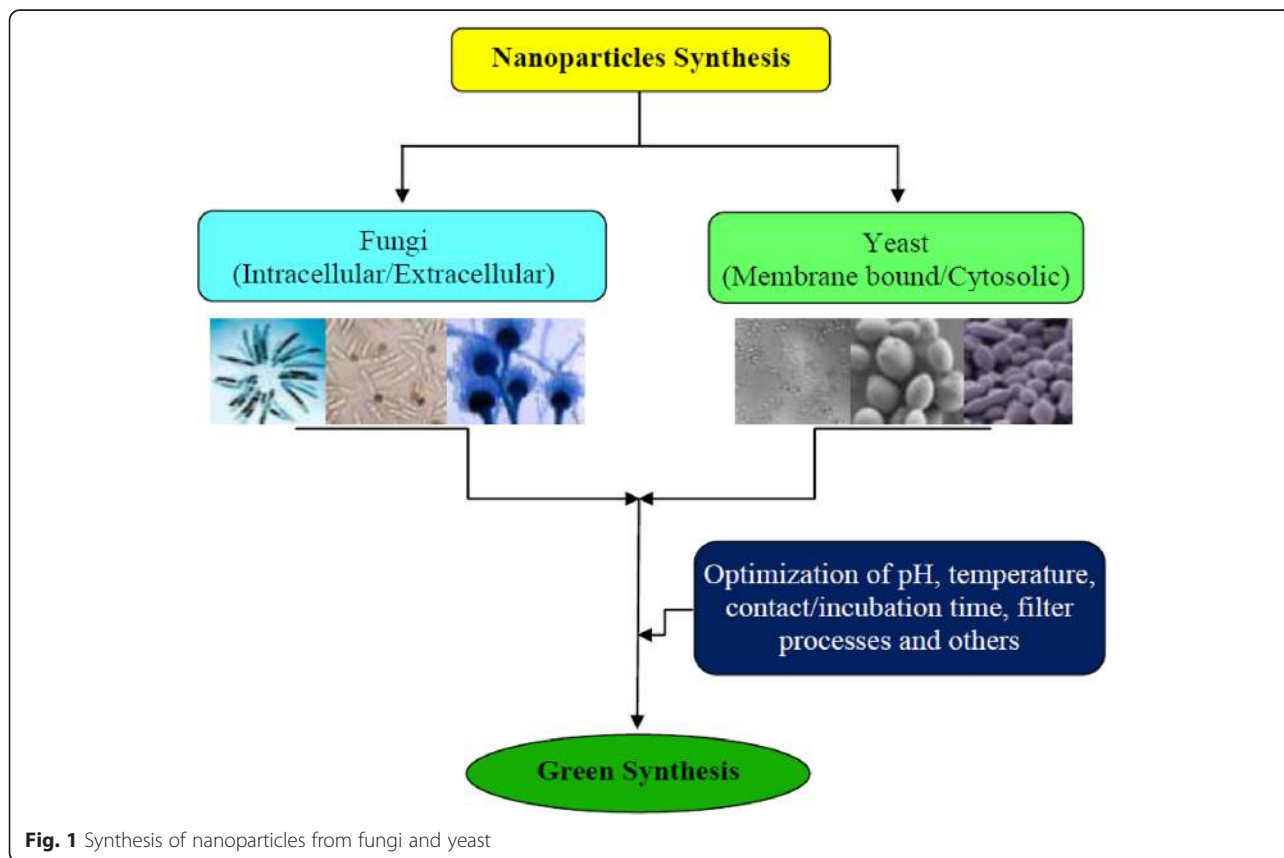
Biogenic synthesis of metal nanoparticles involves bio-reduction of metal salts to elemental metal which may be stabilized by organic molecules present in the microbes such as fungi and bacteria. The other way of producing metal nanoparticles is biosorption where metal ions in the aqueous medium are bonded to the surface of the cell wall of the organisms. For large-

scale production of nanoparticles, fungi and yeasts are preferred over other organisms (Figs. 1 and 2). When fungus is exposed to metal salts such as  $\text{AgNO}_3$  or  $\text{AuCl}_4^-$ , it produces enzymes and metabolites to protect itself from unwanted foreign matters, and in doing so, the metal ions are reduced to metal nanoparticles [91]. The fungi also produce naphthoquinones and anthraquinones [92–95] which act as reducing agents. Thus, a specific enzyme can act on a specific metal. For instance, nitrate reductase is essential for ferric ion reduction to iron nanoparticles. It was reported that for metal ion reduction, not only the enzyme was necessary but also an electron shuttle [14]. It is well understood that nanomaterials may be beneficial or harmful to living systems [1–6]. For example, Cd, Hg, Pb, and Tl nanoparticles are toxic and produce adverse effect in mammals and plants. The toxicity also depends on their shape, size, and the nature of the specific metal ion.

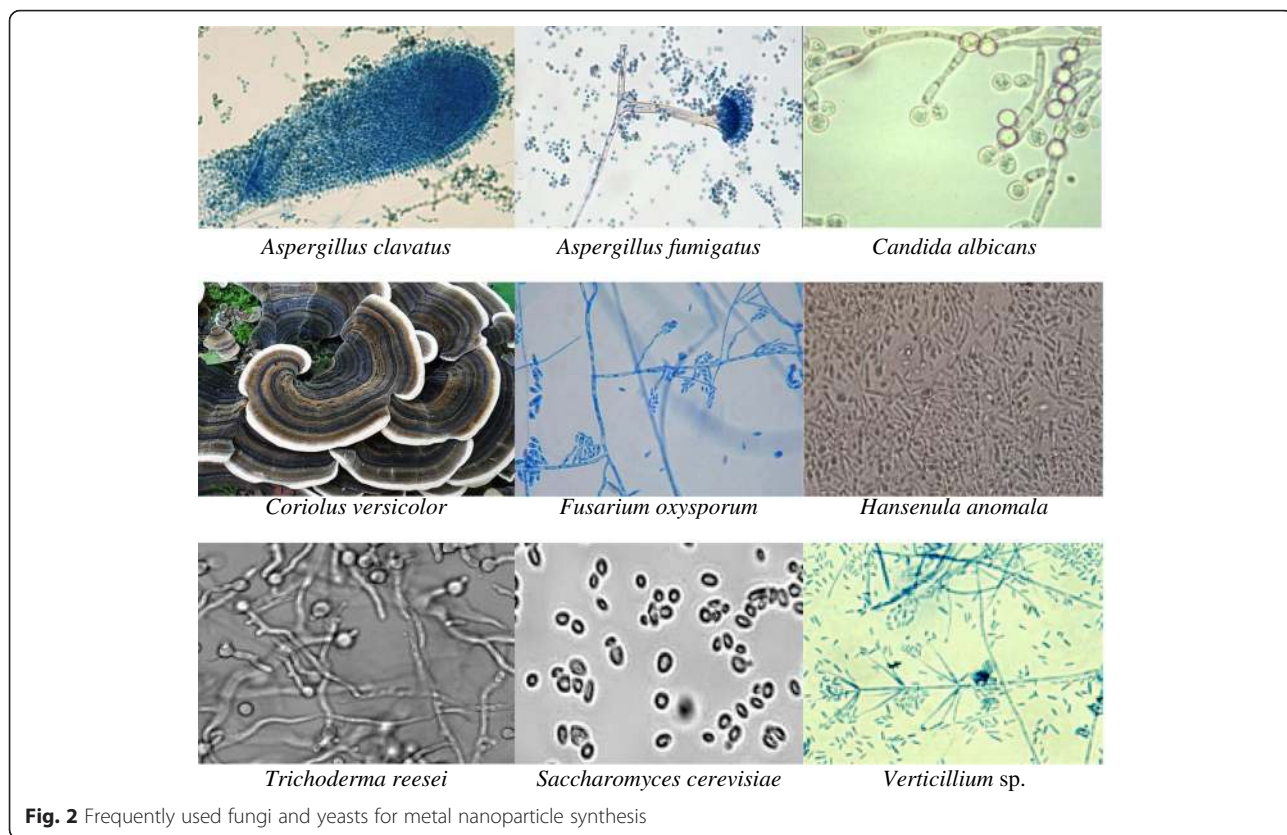
**Silver Nanoparticles**

Major work has been done with silver nanoparticles produced by fungi extracellularly or intracellularly [29, 51]. The particle size is metal and fungi specific. The silver nanoparticles produced from the interaction of *Aspergillus*

*fumigates* may not have the same dimension as those produced by *Fusarium oxysporum* even if the other conditions like concentration, pH, and temperature are identical [29]. The incubation time may also vary from 15 to 60 min [29, 51]. Synthesis of silver nanoparticles from *Trichoderma reesei* takes 72 h, but it is useful for large-scale production of nanoparticles. Their size ranges between 5 and 50 nm [84]. It has also been reported by Vahabi et al. [84] that manipulation of the method can produce enzymes up to 100 g/L which is unprecedented and requires confirmation. Silver nanoparticles of 20–50 nm, obtained from *F. oxysporum*, aggregate in spherical shape (Fig. 3) [14]. In this study, the extracellular reduction of metal ions was done by a nitrate-dependent reductase enzyme and a shuttle quinone. Sanghi et al. [45] studied the extra- and intracellular formation of silver nanoparticles by *Coriolus versicolor*, commonly known as white rot fungus. Extracellular production of silver nanoparticles from fungi *A. fumigates* [29] and *Phoma* sp. [96] has also been reported. In addition, the fungus, *Trichoderma viride*, was used to synthesize polydispersed silver nanoparticles of 5 to 40 nm at about 27 °C which showed an absorption band at 420 nm in UV-visible spectrum [85]. Antibacterial properties were tested against four bacterial strains namely, *Salmonella typhi* (gram-negative rods), *Escherichia coli* (gram-negative rods),

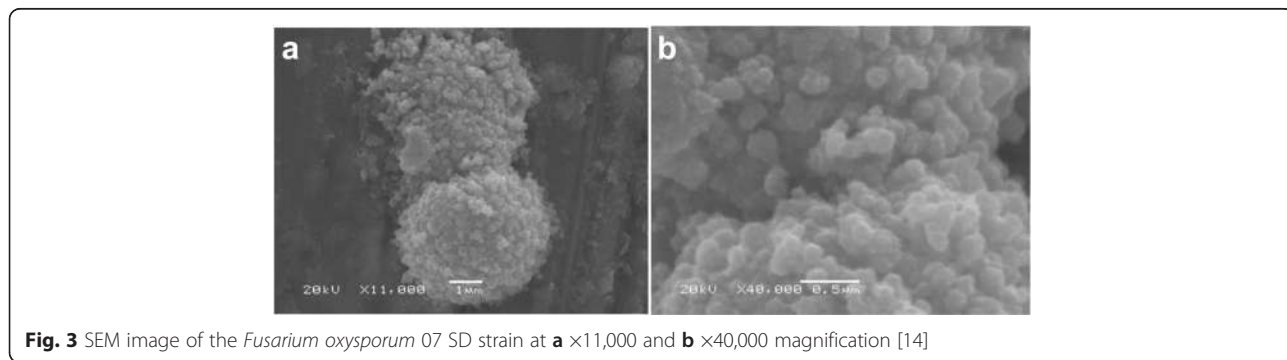


**Fig. 1** Synthesis of nanoparticles from fungi and yeast



*Staphylococcus aureus* (gram-positive cocci), and *Micrococcus luteus* (gram-positive cocci). It was observed that the antibacterial activities of ampicillin, kanamycin, erythromycin, and chloramphenicol were significantly enhanced in the presence of silver nanoparticles. *Geotricum* sp. was found to successfully produce silver nanoparticles with particle sizes ranging from 30 to 50 nm [97]. The fungus *Verticillium* (from *Taxus* plant) has also been used to synthesize silver nanoparticles with average size of  $25 \pm 12$  nm at room temperature [87]. It is noteworthy that silver ions were not toxic to the fungal cells, and they continued

to multiply even after biosynthesis of the silver nanoparticles. Rice husk is a cheap agro-based waste material, which harbors a substantial amount of silica in the form of amorphous hydrated silica grains. Therefore, it would be an ideal material to biotransform amorphous to crystalline silica nanoparticles. Yang et al. [98] have suggested that in such cases, the nanoparticles form complexes. It must be made clear at this stage that only metal ions are bonded to the organic groups by virtue of the positive charge on them and the lone pair of electrons on the organic functional groups. In no case may a neutral metal atom be bonded to any electron-

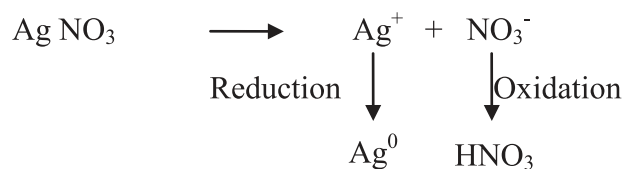


donating molecule. There is always a great deal of confusion about metal ions and metal nanoparticles. A metal ion is a positively charged particle of much smaller size than an electrically neutral metal atom. When the metal ion is bonded to the surface of the fungal cell, it undergoes reduction to form metal nanoparticles with subsequent oxidation of organic molecules whether enzyme, protein, or peptide. It is quite obvious that oxidation and reduction are simultaneous processes.

Kowshik et al. [99] demonstrated the extracellular formation of 2- to 5-nm-long silver nanoparticles by a silver-tolerant yeast strain MKY3. Subramanian et al. [100] reported the effectiveness of marine yeasts (*Pichia capsulata*) derived from the mangrove sediments to synthesize silver nanoparticles (1.5 mM  $\text{AgNO}_3$ , 0.3 % NaCl, pH 6.0, incubated at 5 °C for 24 h) that exhibited an absorption peak at 430 nm.

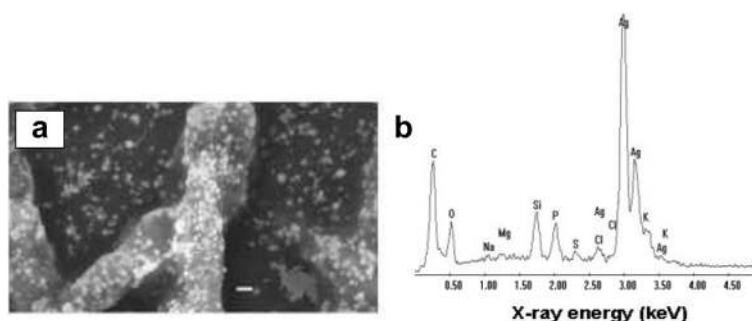
Extracellular biosynthesis and characterization of silver nanoparticles employing *Aspergillus flavus*, *A. fumigates*, *Neurospora crassa*, and *Phaenerochate chrysosporium* have been reported by many workers [29, 66, 101–103]. Nanoparticles of Au-Ag have also been reported [53, 54]. Gericke and Pinches [104] have obtained gold nanoparticles of different shapes and sizes from fungal cultures. It has been observed that their size can be controlled by monitoring concentration, pH, and temperature of the solution. It has also been noted that intracellular synthesis yields nanoparticles of smaller size.

The exact mechanism of intracellular synthesis of gold and silver nanoparticles is not known, but it is for sure that in the presence of fungi, they are formed on the surface of mycelia. It is proposed that the metal ions in the solution are attracted towards fungal mycelia by virtue of the positive charge on them and the slightly negative charge on the cell wall due to carboxylic groups on the enzyme or amino group of the protein, followed by reduction of the metal ions producing metal nanoparticles [105].

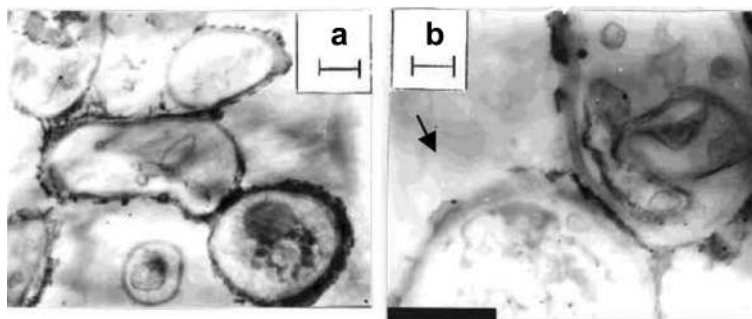


The acidophilic fungus, *Verticillium* sp., isolated from the taxus plant, was allowed to interact with  $\text{AgNO}_3$  solution at 28 °C for 72 h. The transformation was monitored visually and spectroscopically by a change in color of the fungal biomass. Both gold and silver nanoparticle formation were further confirmed by a comparison of their spectra before and after their exposure to the fungi. It is also significant to note that the fungi keep on growing even after the formation of silver nanoparticles, indicating that they are not toxic to the *Verticillium* sp. However, in most of the bacterial species, the growth is arrested showing that Ag/Au nanoparticles are toxic to them. The inhibition of bacterial cell growth in the presence of Ag nanoparticles is assumed to be a defensive mechanism to sequester the metal ions as a consequence of which the  $\text{Ag}^+$  ions are reduced or complexed with proteins in the bacterial cells. The SEM image (Fig. 4a) of *Verticillium* spp. exposed to  $\text{AgNO}_3$  solution for 72 h showed uniform distribution of Ag nanoparticles over the entire surface of the fungal cell [106]. EDAX also indicated an abundance of Ag nanoparticles (Fig. 4b) besides other weak peaks for C, S, P, Mg, and Na. The TEM analysis of the above sample (Fig. 5a, b) displayed scattered dark spots identified as Ag nanoparticles of  $25 \pm 12$  nm.

Li et al. have reported the fabrication of Ag nanoparticles of 1–20 nm from *Aspergillus terreus* in pretty good yields [35]. It is expected that the fungi secrete NADH as one of the components as reducing agents, which along with other ingredients, reduce the metal ions to metal nanoparticles. In order to confirm their hypothesis, NADH alone was added to  $\text{AgNO}_3$  solution which did not show any change in color. However, when



**Fig. 4** **a** SEM image of the *Verticillium* fungal cells after immersion in  $10^{-4}$  M aqueous  $\text{AgNO}_3$  solution for 72 h (scale bar = 1 mm). **b** EDAX spectrum recorded from a film of fungal cells after formation of silver nanoparticles. Different X-ray emission peaks are labelled [106]



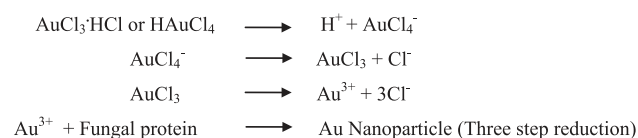
**Fig. 5 a, b** TEM images of thin sections of stained *Verticillium* sp. cells after reaction with  $\text{Ag}^+$  ions for 72 h at different magnifications. Scale bars in **a** and **b** correspond to 1 and 500 nm, respectively [106]

NADH was added along with a fungal extract to  $\text{AgNO}_3$ , the reaction started after a few minutes. It shows that NADH is a key factor in the synthesis of Ag nanoparticles, but other molecules are also essential which perhaps, catalyze the redox reaction. In many microorganisms, NADH is present as a coenzyme such as reductase secreted by *A. terreus*. Since NADH acts as an electron carrier and  $\text{Ag}^+$  ions as electron acceptor, reduction of  $\text{Ag}^+$  to Ag nanoparticle occurs [58, 106]. The Ag nanoparticles were examined for their antimicrobial activity. The results (Table 2) indicated that nanoparticles are broad spectrum antimicrobial agents. In some cases, they are effective even against fluconazole-resistant fungi [107].

### Gold Nanoparticles

Biosynthesis of gold nanoparticles from fungi has been reviewed very recently [17]. They are resistant

to oxidation and dispersed [107] nicely. The color corresponds to the particle size in general. For instance, yellow, red, and mauve refer to large, small, and fine nanoparticles, respectively, of varying size and morphology [108]. It is claimed that gold nanoparticles can be stabilized by substances like ascorbic acid and citrate [109]. Stabilization can also be achieved by polyvinyl alcohol [110]. Enzymes are said to be responsible for the biosynthesis of gold nanoparticles. The intra- or extracellular synthesis of nanoparticles by fungi is done in a simpler manner. The gold ions are trapped by the proteins and enzymes on the surface of the fungi and get reduced. They further form aggregates of large dimensions [111]. The gold nanoparticles synthesized from various sources have different properties. They have been checked for their cytotoxic effects against cancer [69]. Both the intracellular and extracellular reduction of AuCl or  $\text{AuCl}_3$  follow the same pathway [112]. Since AuCl requires one electron to give gold nanoparticles, it follows one-step reduction whereas  $\text{AuCl}_3$  requires three electrons and reduction occurs in three steps. As an example, when  $\text{AuCl}_3$  is dissolved in water, the following reactions occur at the mycelia of fungi which contain proteins, etc. and the metal nanoparticles are produced.



It is to be noted that in the event of intracellular gold nanoparticle formation, the  $\text{Au}^{3+}$  ions being smaller than  $\text{Au}^+$  ions penetrate or simply diffuse into the cell membrane and get reduced there. It is, however, inconclusive if diffusion of  $\text{Au}^{3+}$  ions into the fungal cell occurs through accumulation or absorption. As the concentration of gold nanoparticles increases, the  $\text{Au}^{3+}/\text{Au}^+$

**Table 2** Size of the inhibition zone for AgNPs synthesized by *Aspergillus terreus* against the tested microorganisms [35]

Tested pathogenic organisms	Mean size of inhibition zone (mm)	
	Control	Test
<i>Candida albicans</i> (ATCC 90028)	9	16 ± 1
<i>C. krusei</i> (ATCC 6258)	10	14 ± 2
<i>C. parapsilosis</i> (ATCC 22019)	9	13 ± 1
<i>C. tropicalis</i> (JLCC 30394)	10	14 ± 1
<i>Aspergillus flavus</i> (IFM 55648)	9	13 ± 2
<i>A. fumigates</i> (IFM 40808)	9	14 ± 2
<i>Staphylococcus aureus</i> (ATCC 25923)	9	16 ± 1
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	9	12 ± 1
<i>Escherichia coli</i> (ATCC 25922)	10	13 ± 1

ATCC American Type Culture Collection, USA; IFM Institute for Food Microbiology (at present the Medical Mycology Research Center, Chiba University), Japan; JLCC Culture Collection of Jilin University, Mycology Research Center, China  
Control:  $\text{AgNO}_3$ ; test: AgNPs

concentration falls. Metal nanoparticles induce oxidative stress in fungi and other microorganisms. Higher concentration of metal nanoparticles inhibits growth and protein expression [113] in *Rhizopus oryzae*. It is also likely that for a certain metal ion reduction, a specific type of protein is involved. However, it may be understood from hard and soft acid and base theory (HSAB) that donor acceptor complexation of metal with organic bases may occur.

Narayanan and Sakthivel [80] have demonstrated the formation of gold nanoparticles in the presence of the fungus *Cylindrocadium floridanum*. They noted that in 7 days, the fungi accumulated face-centered cubic (fcc) (111)-oriented crystalline gold nanoparticles on the surface of the mycelia. It was confirmed by the appearance of a characteristic peak at 540 nm in the UV-vis region. The nanoparticles are useful in degrading 4-nitrophenol where the process follows a pseudo-first-order kinetic model with the reaction rate constant of  $2.67 \times 10^{-2} \text{ m}^{-1}$  with  $5.07 \times 10^{-6} \text{ mol dm}^{-3}$  of gold of about 25 nm. As the reaction proceeds, an increase in gold nanoparticle concentration from  $2.54 \times 10^{-6}$  to  $12.67 \times 10^{-6} \text{ mol dm}^{-3}$  occurs with a reduction in size from 53.2 to 18.9 nm.

Mukherjee et al. [87] have reported the formation of gold nanoparticles from *Verticillium* sp. which was found on the surface of mycelia. Gold nanoparticles have also been produced from *Verticillium* fungi. When  $\text{HAuCl}_4$  solution was added to fungal biomass, it started turning purple within a few hours of exposure while the aqueous solution of  $\text{HAuCl}_4$  remained colorless. It indicated intracellular nanoparticle formation. The morphology of Au nanoparticles does not appear to have a relationship with fungal species; as in all cases, several types of nanoparticles are formed [51, 87]. It was observed that old fungal biomass is less effective in producing Au nanoparticles than the fresh ones. It is probably due to a larger secretion of proteins and enzymes in the fresh fungal biomass than the aged ones.

Kumar et al. [64] showed the applicability of yeast species *Hansenula anomala* to reduce gold salt in the presence of amine-terminated polyamidoamine dendrimer as stabilizer. Lim et al. [114] used *Saccharomyces cerevisiae* broth to synthesize gold and silver nanoparticles. Gold nanoparticles of 2- to 100-nm size were prepared at pH 4–6 in 24 h which had an absorption maximum at 540 nm. Extracellular synthesis of silver NP of 10–20 nm was done at pH 8–10 in 48 h. It displayed a characteristic absorption peak at 415 nm. Gold and silver nanoparticles, with face-centered cubic structures prepared from *Candida guilliermondii* [115], exhibited distinct surface plasmon peaks at 530 and 425 nm, respectively. These nanoparticles were tested against five pathogenic bacterial strains. The highest efficiency for both gold and silver nanoparticles was observed against *S. aureus*, which

indicated the applicability of yeast-synthesized nanoparticles for environmental remediation. *Yarrowia lipolytica* was reported to be an effective reducing agent to produce gold nanoparticles and nanoplates by varying concentrations of chloroauric acid at pH 4.5 [90]. According to the findings, a mixture of 109 cells  $\text{ml}^{-1}$  and 0.5 or 1.0 mM of the gold salt developed a purple or golden red color indicating the formation of gold nanoparticles. Nanoparticles of different sizes were obtained by incubating 1010 cells  $\text{ml}^{-1}$  with 0.5, 1.0, or 2.0 mM chloroauric acid. It was confirmed that an increase in salt concentration at a fixed number of cells resulted in the increase of nanoparticles. On the other hand, an increase in cell numbers at a constant gold salt concentration resulted in a significant decrease in nanoparticle size. From Fourier transform infrared spectroscopy (FTIR) spectral data, the presence of carboxyl, hydroxyl, and amide groups on the cell surfaces was confirmed.

Soni and Prakash [116] have reported the green synthesis of gold nanoparticles from *Aspergillus niger* and identified it by a change in color and its absorption at 530 nm. They have also suggested that broadening of the band is due to the aggregation of gold nanoparticles. Perhaps it refers to the low concentration of the nanoparticles because the peak centered at 530 nm will obviously become sharp as a result of the increased quantity of nanoparticles. They have also reported that the Au nanoparticles are toxic to *Anopheles stephensi*, *Culex quinquefasciatus*, and *Aedes aegypti* mosquito larvae. Silver nanoparticles synthesized from *Pleurotus ostreatus* fungi were characterized by UV-vis, SEM, EDS, XRD, and TEM. Silver nanoparticles in solution were identified by the appearance of a peak at 440 nm in the visible region of the spectrum. XRD pattern showed their crystalline nature. The SEM and TEM images showed depressed Ag nanoparticles of nearly 50 nm. Their antimicrobial activity was tested against Gram-positive and Gram-negative bacteria namely, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *S. aureus*, and *Vibrio cholera*. It was observed that Ag nanoparticles are much less effective against the above pathogens relative to the antibiotics, but when antibiotics are fortified with Ag nanoparticles, their activity is enhanced. It is quite likely that a suitable mixture of antibiotic and Ag nanoparticles may be more effective as a medicine for drug-resistant pathogens.

#### Other Metal Nanoparticles

Castro-Longoria et al. [117] have produced platinum nanoparticles and their aggregates using the fungus *N. crassa*. Both intracellular single platinum nanoparticles of 4- to 3-nm diameter and spherical agglomerates of 20- to 110-nm diameter were produced. A comparison of platinum nanoparticles synthesized from biomass was made with those prepared from *N. crassa* extract. It was



**Table 3** List of plant pathogenic fungi (modified from 128)

Fungal species (KACC accession no.)	Common names	Host plants
<i>Alternaria alternata</i> (A-1 40019)	Alternaria leaf blight	Strawberry, pepper, tomato
<i>Alternaria brassicicola</i> (A-2 40857)	Black spot	Cauliflower, radish, cabbage, kale
<i>Alternaria solani</i> (A-3 40570)	Alternaria leaf spot	Pepper, tomato, eggplant, potato
<i>Botrytis cinerea</i> (B-1 40574)	Gray mold	Eggplant, tomato, potato, pepper, strawberry
<i>Cladosporium cucumerinum</i> (C-1 40576)	Scab	Eggplant, cucumber, pumpkin, melon
<i>Corynespora cassiicola</i> (C-9 40964)	Leaf spot	Pepper, cucumber, bean, tomato, sesame
<i>Cylindrocarpon destructans</i> (C-10 41077)	Root rot	Strawberry, ginseng, peony
<i>Didymella bryoniae</i> (D-1 40938)	Black rot	Cucumber, pumpkin, watermelon, melon
<i>Fusarium oxysporum</i> f. sp. <i>Cucumerinum</i> (F-1 40525)	Fusarium wilt	Cucumber
<i>F. oxysporum</i> f. sp. <i>Lycopersici</i> (F-2 40032)	Fusarium wilt	Tomato
<i>F. oxysporum</i> (F-3 40052)	Fusarium wilt	Tomato
<i>Fusarium solani</i> (F-4 41643)	Fusarium wilt	Potato, ginseng
<i>Fusarium</i> sp. (F-5 40050)	Fusarium rot	Potato, sweet potato, pepper, strawberry, pear tree
<i>Glomerella cingulata</i> (G-1 40895)	Anthracoise	Pepper, strawberry, grapevine
<i>Monosporascus cannonballus</i> M-1 40940)	Root rot	Cucumber, pumpkin, watermelon, melon
<i>Pythium aphanidermatum</i> (P-8 40156)	Damping-off	Tomato, tobacco, radish
<i>Pythium spinosum</i> (P-9 41060)	Root rot	Sweet potato, pumpkin, cabbage
<i>Stemphylium lycopersici</i> (S-3 40967)	Leaf spot	Eggplant, tomato, pepper

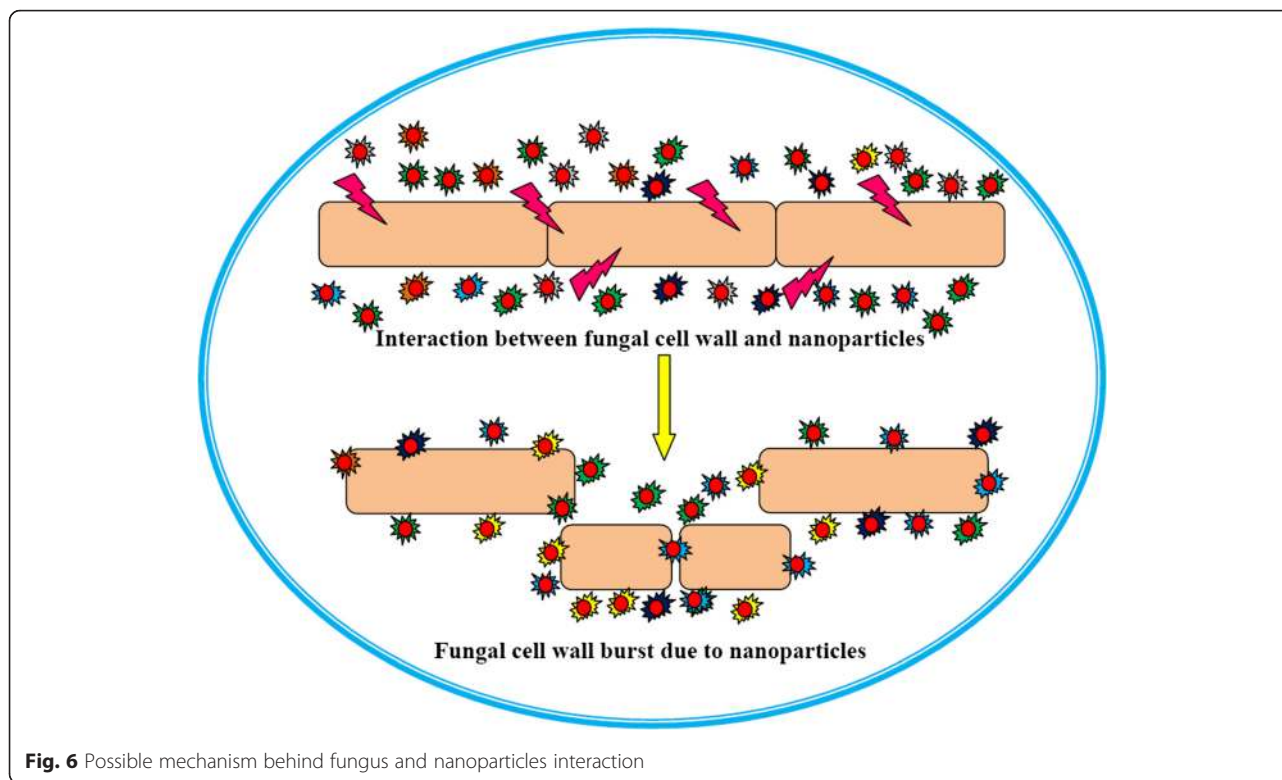
KACC Korean Agricultural Culture Collection, Suwon, Korea

noticed that the platinum nanoparticles produced from the extract were only single crystal nano-agglomerates. However, the quantity of nanoparticles synthesized extracellularly differs significantly from those prepared intracellularly. Magnetite,  $\text{Fe}_3\text{O}_4$  magnetite nanoparticles have been obtained from *F. oxysporum* and *Verticillium* sp. [118]. Extracellular synthesis of fairly smaller selenium nanoparticles of the order of 47 nm from *A. terreus* was also done in 60 min [119]. It was found that *Schizosaccharomyces pombe* and *Candida glabrata* were capable of intracellular production of CdS nanoparticles from cadmium salt in solution [120]. CdS nanoparticle synthesis using *S. pombe* has been considered to be dependent on a stress protein response [99]. Phytochelatins gets activated on exposure of *S. pombe* to cadmium and synthesizes phytochelatin. These phytochelatin chelate the cytoplasmic cadmium to phytochelatins–Cd complex. Thereafter, an ATP-binding cassette-type vacuolar protein transports phytochelatins–Cd complex across the vacuolar membrane. Within the vacuole, sulfide gets added to the complex to form a high-molecular-weight phytochelatins–CdS<sup>2-</sup> complex/CdS nanocrystal.

#### Metal Nanoparticles and Plant Pathogenic Fungi

Fungi are accountable for more than 70 % of all major crop diseases [121]. The annual crop losses due to pre- and post-harvest fungal diseases exceed 200 billion euros, and in the USA alone, over \$600 million are annually spent on fungicides [122]. Impact of nanoparticles

on crop plants is a rising area of research that needs to be meticulously explored. In recent years, engineered nanoparticles have achieved particular attention as a potential candidate for improving crop yield, resistance, and disease management technologies [5, 6, 123]. However, these applications are still in their infancy. This is simply due to the unprecedented and unforeseen health hazards and environmental concerns [6]. It is understood that the use of pesticides in agriculture is becoming more hazardous day by day. In order to replace such toxic materials by equally useful substances is an excellent choice, especially easily available silver nanoparticles which are antimicrobial for most of the fungal and bacterial diseases in man and plants [124, 125]. Jo et al. [126] have reported antifungal activity of Ag<sup>+</sup> ions and Ag nanoparticles on pathogenic fungi. It is useful if a better protocol for its application in plants is developed. The Ag nanoparticles in aqueous medium catalyze complete destructive oxidation of microorganism [127]. Kim et al. [128] have studied the growth inhibition effect of three types of Ag nanoparticles against 18 different plant pathogenic fungi in vitro (Table 3). A variety of host plants including the cucumber family, tomato, potato, and cabbage, which are very prone to infections, have been treated. It has been noted that growth inhibition is concentration-dependent and the most effective concentration leading to complete destruction of fungi is 100 ppm. Possible mechanism of interaction between fungi and nanoparticles is presented in Fig. 6.



**Fig. 6** Possible mechanism behind fungus and nanoparticles interaction

Silver nanoparticles were used as an alternative to pesticides to control the sclerotia-forming phytopathogenic fungi [129]. The antifungal effect of doubly encapsulated silver nanoparticle solution against rose powdery mildew (leaf distortion, leaf curling, early defoliation, and reduced flowering) caused by *Sphaerotheca pannosa* var *rosae* was also studied [130]. A 10-ppm silver nanoparticle solution of 1.5 nm was sprayed over a large area infected by rose powdery mildew. After 2 days, more than 95 % of them faded out and did not recur for a week. The toxic effect of the silver nanoparticles of 5–24 nm on *Colletotrichum gloesporioides*, which causes anthracnose in a wide range of fruits, such as apple, avocado, mango, and papaya has also been studied [131]. A significant delay in growth of *C. gloesporioides* was observed. Silver nanoparticles may, therefore, be used as an alternative to fungicides for plant disease management. Das et al. [132] have reported extracellular synthesis of gold nanoparticles of 10 nm from *R. oryzae* which was employed for the generation of nanogold-bioconjugate structure. These nanostructures displayed excellent adsorption capacity and were successfully employed to purify water free from pathogens and pesticides.

#### Application of Metal Nanoparticles

There are a myriad of applications of metal nanoparticles such as cosmetics, catalysts, lubricants, fuel additives, paints, agro-chemicals, food packaging, textile engineering,

electronics, optics, environmental sensing, nanomedicine, drug and gene delivery agents, biodegradation of pathogens, tumor destruction via heating (hyperthermia), magnetic resonance imaging, and phagokinetic studies [2–9, 23, 24, 133–136, 139]. Fungus-mediated synthesis of metal nanoparticles is getting much attention due to their extensive application in various sectors (Table 4). Durán et al. [141] have reported that silver nanoparticles (1.6 nm) obtained extracellularly from *F. oxysporum* can be incorporated in clothes which can prevent infection from *S. aureus*. Silver nanoparticles (1–10 nm) attach to the bacterial cell surface and significantly disrupt its respiration and permeability [154]. Silver nanoparticles (5–25 nm) from *A. fumigates* were produced to understand the biochemical and molecular mechanism of synthesis [29]. The ability of the fungi, *F. oxysporum*, to hydrolyze metal complexes demonstrates the formation of metal oxide semiconducting materials [59]. Namasivayam and Avimanyu [143] have reported that when the silver nanoparticles of 45–100 nm obtained from *Lecanicillium lecanii* were coated on the bleached cotton fabrics using acrylic binder, they became resistant to *S. aureus* and *E. coli* infection. A method was invented to decorate the growing fungal hyphae of *A. niger* with a high load of gold nanoparticles, which were initially produced using aqueous tea extract as a sole reducing/stabilizing agent [155]. Heat treatment of these hybrid materials yielded porous gold microwires. It is anticipated that the nanowire-based paper may be used to

**Table 4** Applications of metal nanoparticles synthesized by fungi and yeasts

Nanoparticle	Fungi/yeasts	Application	References
Ag	<i>Alternaria alternata</i>	Enhancement in antifungal activity of fluconazole against <i>Phoma glomerata</i>	[136]
	<i>Aspergillus clavatus</i>	Antimicrobial activity	[137]
	<i>A. niger</i>	Antibacterial activity	[138]
	<i>A. niger</i>	Wound healing activity	[131]
	<i>Colletotrichum gloeosporioides</i>	Antifungal activity	[141]
	<i>Fusarium acuminatum</i>	Antibacterial activity	[140]
	<i>F. oxysporum</i>	Textile fabrics	[141]
	<i>F. solani</i>	Textile fabric	[142]
	<i>Lecanicillium lecanii</i>	Textile fabrics	[143]
	<i>Macrophomina phaseolina</i>	Antimicrobial properties against multidrug-resistant bacteria	[144]
	<i>Penicillium oxalicum</i>	Catalytic activity	[145]
	<i>Penicillium</i> sp.	Antibacterial activity against MDR <i>E. coli</i> and <i>S. aureus</i>	[146]
	<i>Phytophthora infestans</i>	Antimicrobial activity	[147]
	<i>Pleurotus ostreatus</i>	Antimicrobial activity	[148]
	<i>Raffaelea</i> sp.	Antifungal activity	[149]
	Au	<i>Trichoderma crassum</i>	Antimicrobial activity
<i>T. viride</i>		Vegetable and fruit preservation	[151]
<i>Aspergillus japonicus</i> AJP01		Catalytic activity	[152]
Cds	<i>A. niger</i>	Toxic to mosquito larvae	[116]
	<i>Rhizopus oryzae</i>	Water hygiene management	[132]
Cds	<i>Saccharomyces pombe</i>	Electric diode	[78]
	<i>F. oxysporum</i>	Live cell imaging and diagnostics	[153]

clean up oil and organic pollutants in water and soil sediments. Nanofibrous mats were prepared by Spasova et al. [156], which contained chitosan and *T. viride* spores. It was reported that *T. viride* kept at 28 °C grows much faster and fights for space and nutrients against *Fusarium* sp. and *Alternaria* sp. Moreover, *T. viride* produces extracellular hydrolytic enzymes which directly attack the pathogen and destroy their cell walls. Advances in luminescent nanocrystals have led to fluorescent labelling by QDs with bio-recognition molecules [157]. When *F. oxysporum* was incubated with a mixture of CdCl<sub>2</sub> and SeCl<sub>4</sub>, highly luminescent CdSe quantum dots were produced at room temperature (26 ± 1 °C) [153]. In addition, nitrate reductase from *F. oxysporum* has been shown to catalyze the production of stable silver nanoparticles in vitro. It suggests the way for designing a rational enzymatic strategy for the synthesis of nanomaterials of different composition, shape, and size [158]. An optical sensor for the detection of pesticides (Siven 85 % wettable powder) in water using ZnCdSe QD films has also been developed [159].

## Conclusions

It is an established fact that biogenic synthesis of metal nanoparticles by fungi is a safe and economical process

because stable and small-sized nanoparticles are generally produced. Their role in drug delivery, magnetic resonance imaging, catalysis, environmental sensing, textile engineering, food sectors and plant disease management is well known. Several precious metals may be easily recovered from large heap of wastes containing metal salts. This process of producing nanoparticles by a redox process may be employed to produce pure metals. The fungi may therefore be used in metallurgical operations to sequester metal from ores. It can save time and money. Since some of the metal ions are toxic to many microbes, they can be used as a prophylactic to inhibit their growth. However, a comprehensive protocol may be developed to control the morphology of metal nanoparticles for their application in all sectors of medicine, agriculture, and technology.

## Competing Interests

The authors declare that they have no competing interests.

## Authors' Contributions

AH gathered the research data. AH and KSS analyzed these data findings and wrote this review paper. Both authors read and approved the final manuscript.

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