NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 7, ISSUE 11, Nov. -2021

BIOSYNTHESIS OF GOLD NANOPARTICLES FROM FUNGI

Pallavi Gawai
Department of botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad

Ajay Zinzade
Department of botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad

Radha Ghuge
Department of botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad

Shrikant B. Mane
Department of botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad

ABSTRACT:

All selected five fungi Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus. Aspergillus carbonarius and Penicillium citrinum are used for the biosynthesis of gold nanoparticles fresh cell-free filtrate was used for the synthesis of gold nanoparticles. chloroauric acid was the reductant agent mixed with cell-free filtrate of fungi. During the visible analysis, Aspergillus paraciticus biomass was changed from pale yellow to purple color, in Aspergillus carbonarius biomass was changed from black color to Purple and Penicillium citrinum pale brown color to brown color and UV-Visible spectroscopic analysis. In UV-visible spectrum, no peak formation was observed in cell-free extract Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus, Aspergillus carbonarius, and Penicillium citrinum before immersion of $HAuCl_4$ in series 3 and 4, while as strong surface plasmon resonance (SPR) peak of the cell-free extract with $HAuCl_4$ was observed at 450 and 550 nm.

Keywords: Gold nanoparticle, Biosynthesis, $HAuCl_4$

INTRODUCTION:

Gold nanoparticles (Au NPs) are unique compared to other metals from ancient times. It is a highly precious metal in ornament as having used for medicinal purposes for the treatment of a variety of diseases such as smallpox, skin ulcers, measles, and syphilis (Daniel et al., 2004). The term 'nano' comes from the Greek word meaning 'dwarf' i.e., small things in the science and technology language is called the small things called nanotechnology and defined asthe branch of science deals with the study of various aspects of research and technology of the small things. The concept of nanoscience nanotechnology was firstly used by great physicist Richard Feynman at an American physical society meeting at the California Institute of Technology (Cal Tech) on 29 th December 1959. Now today nanotechnology takes very much grip in research and is highlighted inthe world. Nanomaterials (NM) are defined as materials with at least one external dimension in the size range from approximately 1-100 nanometers. A focused integration of bio and nano techniques for biological synthesis of NM, known as bionanotechnology, has emerged from nanotechnology(Kaushik et al 2010).'' The main objective of nanotechnology is the apply nanotools to biological or medical problems and used in various applications

such as imaging (Waren and Nie,1998), sensing (Vascashta et al.,2005), targeted drug delivery (Langer, 2001), gene delivery system (Roy et al.,1999) and artificial implants (Sachlors et al.,2006). It is the intersection of nanotechnology and biology. Nanotechnology combines biological principles with the physical and chemical procedures to generate nano-sized particles with a specific function. It is the proper alternative for the chemical and physical method of nanoparticles formation. It describes the biological system for the production of nanoparticles. Nanoparticle research has great scientific interest due to much potential application in biomedical, optical, and electronic fields.

NANOPARTICLES PRODUCED BY PHYSICAL, CHEMICAL, AND BIOLOGICAL METHOD:

Microbes like bacteria, fungi, yeast, algae, actinomycetes, and higher plant synthesis have the potential to produce silver (Ag), gold (Au), cadmium sulfate (CdS), zink sulfate (ZnS), platinum (Pt), and palladium (Pd) nanoparticles. Among the different groups of micro-organism, fungi are the most effective green route in the synthesis of metal nanoparticles and large scale production, because fungi secret large amounts of proteins and enzymes reduce the metal ion and grow productivity, a large amount of biomass production, and fungi have very high wall binding capacity. Nanoparticles synthesis from fungi known as my nanotechnology which has great demand. Due to their monodispersity, today fungi are bio-nano factories of various metal nanoparticles like silver (Ag), gold (AU), platinum (Pt), cadmium sulfate (CdS). Gold nanoparticles (Au NPs) are unique compared to other metals from ancient time Au-based materials used for medicinal purposes for the treatment of a variety of diseases such as smallpox, skin ulcers, measles, and syphilis (Daniel and Didier, 2004). The gold nanoparticle has antibacterial properties against Staphylococcus aureus and pseudomonas aeruginosa (Duran et al., 2007). Nanoparticles have multifunctional used in various branches. Therefore biological synthesized Nanoparticles have a tremendous amount of application than the chemically synthesized nanoparticles. Has been of great interest. These microbial processes have opened up new opportunities for us to explore novel applications such as a type of bottom-up new approach that has become an attractive focus in current and future generations.

Last few years chemicals methods are used for the production of large quantities of nanoparticles which are complicated, outdated, and expensive which produce hazardous toxic wastes that are harmful to the environment as well as human health. At that time green approach come together which is eco-friendly called as 'Biogenic approach' means nanoparticle synthesis by biological method without the addition of any reducing agent and the stabilizer which are replaced by molecules produced by living organisms i.e. bacteria, fungi, yeast, algae higher plants, etc. Some well-known examples include the use of bacteria, fungi, and plants for the production of nanoparticles. Accumulation of metal ions by microbes has been regarded as low-cost, eco-friendly, and easily achievable with some insights into the future of the hybrid field of more futuristic goals of bio-nanotechnology, such as multifunctional nano-devices, will take much longer to develop in the clinical field (Chan 2006). Fungi were used production of metal nanoparticles. Since fungi have several advantages over bacteria, they are often preferred. Some of the advantages of fungal sources for the production of metal nanoparticles include high tolerance towards metals, high wall-binding capacity, can be easily scaled up, ease to culture on a large scale, and ability to secrete a large number of enzymes (Zeinab et al 2011). Kuber and Dsouza (2006)

reported the use of the fungus Aspergillus fumigatus for the extracellular biosynthesis of silver nanoparticles. The gold nanoparticle has antibacterial properties against Staphylococcus aureus and pseudomonas aeruginosa (Duran et al., 2007). Nanoparticles have multifunctional used in various branches. Therefore biological synthesized Nanoparticles have a tremendous amount of application than the chemically synthesized nanoparticles. Has been of great interest. These microbial processes have opened up new opportunities for us to explore novel applications such type of bottom-up new approach has become an attractive focus in current and future generations. Nanoparticles can be synthesized from physical and chemical methods. The simplest method for the production of nanoparticles is the reduction of their respective salts Aspergillus terreus, which has been used by Rashmi and Preeti (2009) for the biomimetic synthesis and characterization of protein capped silver nanoparticles. A Greener synthesis of nano gold-biocomposite by fungus Cylindrocladium floridanum was reported by Kannan and Natarajan (2011). C. floridanum accumulated gold nanoparticles on the surface of the mycelia when it was cultured in static conditions for 7 days. Thoomatti and Peramchi (2011) used Fusarium oxysporum for both intracellular and extracellular production of gold nanoparticles. The rapid reduction of metal ions resulting in the formation of stable silver and gold nanoparticles of variable size and shape were reported. Greener synthesis of anisotropic nanostructures and isotropic spherical gold nanoparticles using the cell-free filtrate of fungus Sclerotium rolfsii was reported by Kannan and Natarajan (2011). Gold nanoparticles produced by Aspergillus fumigatus were in the size range of 85·1–210 nm and were found to be spherical and had irregular morphologies which were confirmed by SEM analysis. The presence of the gold nanoparticles was confirmed by EDS analysis. The presence of functional groups was confirmed by FT–IR analysis with the peaks in the range of 350–3650 cm−1. The different functional groups associated were found to be C=O, C–N, N–H, O–H. These bonds were found to provide stability to the produced nanoparticles by capping them. XRD results showed that Bragg's reflection was by gold nanoparticles with 2θ = 37·8. Thus, A. fumigatus was found to be a good candidate for the production of gold nanoparticles Pranav et al. (2013). Considering the importance of this fact present topic has become selected for research entitled "**Biosynthesis of Nanoparticles from Microbes**" with the following scheme of working for research.

MATERIAL AND METHODS:

Collection of fungi

Fungi were collected from different abnormal seeds and deteriorated plat parts of differentlocalities of Marathwada and Vidarbha (Buldhana dist). Incidence of fungi from different deteriorated plant parts**,** Naturally infected plant parts leaves and fruits were collected from different market places, botanical gardens, storehouse, and field. Infected plant parts were cut into small pieces; wash with sterile distilled water inoculated on CZA media at 30^0c for seven days.

A). Seed sample

The method described by Neergaard (1973) has been adopted for the collection of seed samples. Accordingly, seeds with different abnormalities were collected from storehouses and markets from different parts of Dist. Aurangabad, Jalna, Beed, and Buldhana regions of Maharashtra state. These seeds were then packed in pre-sterilized polythene bags and kept in laboratory conditions until use.

B). Deteriorated plant parts

Different deteriorated parts of plant parts like leaves and fruits were collected from the garden, market, fields. Infected parts wash

with sterile distilled water and cut into small pieces. Cut pieces preserve at laboratory conditions employed for further experiment.

AGAR PLATE METHOD:

In this method, pre-sterilized corning glass Petri plates of 10 cm diameter were poured with 20 ml of autoclaved Czapek Dox Agar (CZA) medium on cooling the medium, 1 ml serial dilution of composite seed sample, 10 seeds per Petri plates and cut pieces of infected plant parts were incubated in CZA aseptically at 25+-2^0 C. On the seventh day of incubation, the sample was examined under the stereoscopic microscope for the preliminary determination of fungal growth.

Composition of media used in isolation

POTATO DEXTROSE AGAR (PDA):

Peeled potato -200gm, Dextrose -20 gm, Agar – 20 gm. Distilled water – 1000ml, pH -5.6 peeled potatoes were boiled unit soft and passed through a muslin cloth. Then dextrose and agar were added to it and the final volume of solution was made up to 1000 ml, pH was adjusted to 5.6.

Capex Dox Agar (CZA):

Sucrose-30 gm, NANO3-2.0 gm, KH2PO4-1.0gm, MgSO4.7H2O-0.5g, Agar-20gm, and Distilled water 1000 ml, pH-5.6.

Glucose Nitrate (GN):

Glucose – 10 gm, KNO3-2.5 gm, KH2PO4-1.0 gm, MgSO4.7H2O -0.5 gm, and Distilled water -1000 ml, pH-5.8.

NANOPARTICLE PRODUCTION

Production of Biomass

Nanoparticle production was studied by growing the fungi on a liquid GN medium. 100 ml of GN medium was poured in 250 ml conical flasks and autoclaved at 15 lb pressure in 250 ml conical flasks were inoculated separately with 1ml spore suspension of the fungi which were grown for seven days on PDA slants. Unless otherwise stated, the flasks were incubated for 6days at 30^0 C on a rotary shaker (150 rpm). On the seventh day, the flasks were harvested by filtering the biomass through pre-weight Whatman filter paper No.1 and these were extensively washed with sterile distilled water to remove any medium component. The fungal biomass is used for the preparation of cell-free extract (Binupriya et al., 2010; Duran et al., 2007).

SYNTHESIS OF NANOPARTICLES:

Fresh and clean biomass were taken in an Erlenmeyer flask containing 100 ml double distilled water and incubated at 26^{0}C with shaking on a rotary shaker (150 rpm) for 72 hours. After then the biomass was filtered again with Whatman filter paper No .1 and the cell-free filtrate was collected in flak. Silver nitrate, Chloroauric acid most widely used as the source of Gold ions. 1 mM of the aqueous solution of Chloroauric acid (HAuCl4) was used for the synthesis of gold (Au) nanoparticles respectively. 10 ml of Choloroauric acid was mixed with 10 ml of cell-free filtrate of fungi in the Erlenmeyer flask separately. These flasks were incubated at room temperature in dark conditions to minimize the photoactivation of the metal ion. Control (without metal ion) was also run along with the experimental flask.

Based on the color change in the cell-free filtrate after the exposure of metal ions, the fungus is considered a nanoparticle producer. If the color of cell-free filtrate remains as it is after inoculation of metal ions then fungus is considered as non-producer of nanoparticles. In addition to this among the fungi which were screened for nanoparticle production some could notrelease colored matter in the cell-free extract but their mycelial structures were found to be colored after exposure to metal ions.

CHARACTERIZATION OF NANOPARTICLES:

Gold (Au) nanoparticles are

characterized by two analytical techniques as below.

a. Visual analysis of nanoparticles

Visual analysis of nanoparticles is the primary confirmation of the presence or absence of nanoparticles. Silver (Ag), Gold (Au) and Copper (Cu) nanoparticles show specific color after the exposure of metal ions into the cell-free extract. The dark color indicates a large number of nanoparticles while the faint color indicates a less amount of nanoparticles. For externally synthesized silver nanoparticles the silver ion solution generally becomes brownish (Basavraja et al., 2008, Duran et al., 2007). For external gold particle production, the color of the solution can vary depending on the size of the gold nanoparticle. Smaller particles appear pink while large particles appear purple (Vahabi et al., 2011; Shankar et al, 2003).

b. UV –spectrophotometric analysis

A spectrophotometer was invented by Arnold (2004) and is one of the most essential instruments which covered both visible and ultraviolet spectrums. A spectrophotometer determines the composition of solution i.e. concentration of certain substances within a solution by measuring how the solution absorbs a particular wavelength of light. UV-Vis spectrophotometers allow the identification, characterization, and analysis of metallic nanoparticles like silver, gold, and copper. They can be evaluated dispersion and local structure of nanoparticles and also used to confirm the metallic nature, size, and aggregation level.

RESULT AND DISCUSSION:

Table no. 1. Selected fungi for Gold Nanoparticle synthesis

Sr. No	Selected fungi	Code
1	Colletotrichum fulcatum	A
2	Trichoderma atroviride	B
3	Aspergillus paraciticus	C
4	Aspergillus carbonarius	D
5	Penicillium citrinum	E

During the investigation, study fungi were isolated from different sourness like seed and different deteriorated parts of plant parts like leaves and fruits among the isolated Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus. Aspergillus carbonarius and Penicillium citrinum fungi were selected these five fungi for further detailed study of nanoparticles production and given codes as in table no. 1. It was clear from table no 2 and figure no1 Colletotrichum fulcatum produce Ash color of mycelium with Yellow culture filtrate (C.F), Trichoderma atroviride Yellow-green color of mycelium with Brown culture filtrate (C.F), Aspergillus paraciticus Pale yellow color of mycelium with Pale yellow culture filtrate (C.F), Aspergillus carbonarius black color of mycelium with whitish culture filtrate (C.F), and Penicillium citrinum Yellow-green color of mycelium with Pale brown culture filtrate (C.F).

Table no 2. Growth and production Crude cell filtrate of different selected fungi

Sr. No.	Code of selected fungi	Colour of Mycelium	Culture filtrate (C.F)
1	A	Ash	Yellow
2	B	Yellow green	Brown
3	C	Pale yellow	Pale yellow
4	D	Black	Whitish
5	E	Yellow-green	Pale brown

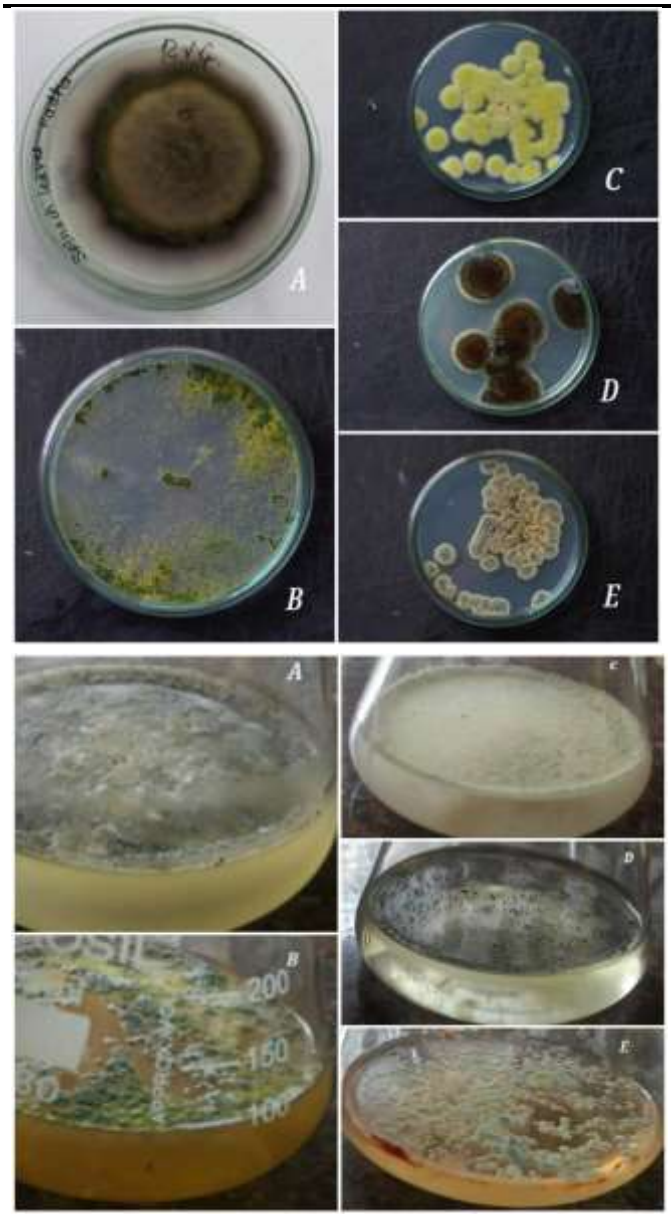


Figure no. 1 Growth pattern of selected fungi on Solid and Broth Media

PRODUCTION OF NANOPARTICLES:

All selected five fungi are used for the production of gold nanoparticles. GN Media is used for the production of biomass of all fungi. They are showing a distinct mycelium structure. Extracellular or intracellular nanoparticle synthesis occurred in fungi with chloroauric acid ($HAuCl_4$) reductant agent. Selected fungi like Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus, Aspergillus carbonarius, and Penicillium citrinum were inoculated separately in 100 ml GN media. After 6 days each flask showed the growth of mycelium.it was clear from table 3 and figure no. 2 biosynthesis of gold nanoparticles Fungal isolated clean, the fresh cell-free filtrate was used for the synthesis of gold nanoparticles. chloroauric acid was the reductant agent mixed with cell-free filtrate of fungi. During the visible analysis, Aspergillus paraciticus biomass was changed from pale yellow to purple color, in Aspergillus carbonarius biomass was changed from black color to Purple and Penicillium citrinum pale brown color to brown color The important thing notes that the color of control was not changing which runs along the experimental flask similar result was found by Pranav et al. (2013). On mixing the fungal biomass with the aqueous solution of chloroauric acid, the color of the biomass was changed from yellow to purple. The color change indicated the reduction of the chloroauric acid ions by the fungal enzyme, which resulted in the formation of gold nanoparticles.

Control **Biosynthesized Gold nanoparticles**

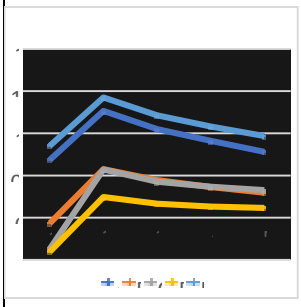
Figure no. 2. Visual analysis Crude cell filtrate of different fungi by adding $HAuCl_4$ solution

Table No. 3. Color of sample Crude cell filtrate of different fungi by adding $HAuCl_4$ solution

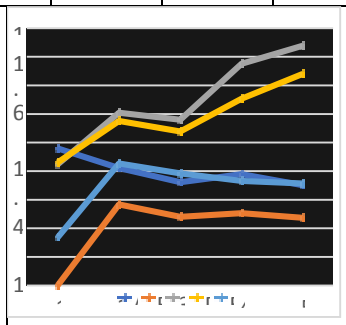
Selected fungi	**Color of cell filtrate after adding $HAuCl_4$**
Colletotrichum fulcatum	white
Trichoderma atroviride	white
Aspergillus paraciticus	purple
Aspergillus carbonarius	Purple
Penicillium citrinum	brown

Table No. 4. Spectroscopic analysis of a cell-free filtrate of the different filamentous fungi in different wavelength

Sr. No.	**Code of fungi**	**Different wavelength**				
		350	400	450	500	550
1	A	1.041	1.73	1.578	1.402	1.242
2	B	0.301	1.071	0.861	0.683	0.559
3	C	0.853	1.428	1.215	1.083	0.969
4	D	-0.398	0.247	0.169	0.135	0.119



Graph 1: Spectroscopic analysis of cell-free filtrate of the different filamentous fungi in different wavelength



Graph 2: Effect of different wavelengths on gold nanoparticle production in fungi.

Table 5: Spectroscopic analysis of the gold nanoparticles obtained using a cell-free filtrate of the different filamentous fungi in different wavelength

Sr. No.	Code of Fungi	Different Wavelength				
		350	400	450	500	550
1	A	0.96	0.824	0.725	0.781	0.704
2	B	0.001	0.568	0.481	0.507	0.474
3	C	0.849	1.211	1.162	1.553	1.68
4	D	0.863	1.154	1.08	1.309	1.484
5	E	0.339	0.856	0.787	0.734	0.716

UV-VIS SPECTROPHOTOMETER ANALYSIS:

All selected five fungi Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus. Aspergillus carbonarius and Penicillium citrinum are used biosynthesis for the production of gold nanoparticles fresh cell-free filtrate was used for the synthesis of gold nanoparticles were monitored by UV-Visible spectroscopic analysis. In UV-visible spectrum, no peak formation was observed in cell-free extract Colletotrichum fulcatum, Trichoderma atroviride, Aspergillus paraciticus, Aspergillus carbonarius, and Penicillium citrinum before immersion of $HAuCl_4$ in series 3 and 4, while as strong surface plasmon resonance (SPR) peak of the cell-free extract with $HAuCl_4$ was observed at 450 and 550 nm which indicates the formation of gold nanoparticles similar result were found by Shelar and Chavan (2014) and gradual change in the color from crystal violet to pink-red with the increase in the concentration of gold chloride due reducing enzymes secreted by the fungus into the surrounding medium might be responsible for the reduction of chloroauric ions. It is well known that gold nanoparticles produce a wide range of colors, due to the excitation of the surface plasmon resonance Mulvaney P. (1996).

REFERENCES:

1) Arnold Orville Beckman. 2004. Biography of

Arnold Orville Beckman. April 10, 1900, Cullom, Illinois, U.S.A d. May 18, La Jolla, California, U.S.A.

2) Arya, V. (2010). Living Systems: Ecofriendly Nanoparticles. Digest Journal of Nanomaterials and Biostructures. 5(1): 9-21.
3) Bala, M. and Arya, V. (2013). Biological Synthesis of Silver Nanoparticles from Aqueous extract of endophytic fungus Aspergillus, Fumigatus and its antibacterial action. International journal of Nano. and Biostructures. 3 (2): 37-4.
4) Bansal, V., Raustray, D-, Ahmad, A., Sastry, M. (2004). Biosynthesis of Zirconia Nanoparticles using the fungus Fusarium oxysporum. Journal of Material Chemistry, 15;2583-2589.
5) Barnett H.L and Bary Hunter. (1998). Illustrated Genera of Imperfect Fungi, IV edition. Published by APS Press St. Paul, Minnesota.
6) Binupriya AR, Sathishkumar M, Yun SI (2010) Biocrystallization of silver and gold ions by inactive cell filtrate of Rhizopus stolonifer. Coll Surf B Biointerf 79:531–534
7) Chan Warren C. W. 2006, Bionanotechnology Progress and Advances Biology of Blood and Marrow Transplantation 12:87-91.
8) Daniel Marie-Christine and Didier Astruc 2004, Gold Nanoparticles: Assembly, Supramolecular Chemistry, Quantum-Size-Related Properties, and Applications toward Biology, Catalysis, and Nanotechnology, Chemical Reviews, Vol. 104, No. 1.
9) Duran Nelson, Marcato Priscyla D., Souza Gabriel I. H. De, Alves Oswaldo L., and Esposito Elisa 2007, Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment, Journal of Biomedical Nanotechnology, Vol.3, 203–208,
10) Shankar, S., Absar, A., and Murali, S. (2003). Geranium leaf assisted biosynthesis of silver nanoparticles. BiotechnolProg 19:1627-1631.
11) Shelar Gitanjali B. and, Ashok M. Chavan 2014. Biological Synthesis and Antibacterial Activity of Silver and Gold Nanoparticles produced by the fungus Aspergillus terreus, International Journal of Science and Research (IJSR)., Volume 3 Issue 6, pp 1679-1683.
12) I.S.T.A. 1966. Proc. International Seed Testing Association. 31:1-52.
13) Kannan Badri Narayanan & Natarajan 2013, Sakthivel Mycocrystallization of gold ions by the fungus Cylindrocladium floridanum, World Journal of Microbiology and Biotechnology volume 29, pages 2207–2211.
14) Kuber C.Bhainsa and Dsouza S. F. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus Aspergillus fumigatus Colloids and Surfaces B: Biointerfaces, Volume 47, Issue 2, Pages 160-164
15) Langer R. 2001. Drugs on target, Science, 293, p. 58
16) Mulvaney P. 1996. Surface plasmon spectroscopy of nanosized metal nanoparticles. Langmuir 12: 788-800.
17) Neergaard, P. 1973. Detection ofseed-borne pathogen by culture tests. Seed Sci. and technol. l : 217-254.
18) Pranav Vasanthi Bathrinarayanan, Dilliganesh Thangavelu, Vasanth Kumar Muthukumarasamy, Chamundeeswari Munusamy, and Baskar Gurunathan 2013 Biological synthesis and characterization of intracellular gold nanoparticles using biomass of Aspergillus fumigatus. Bull. Mater. Sci., Vol. 36, No. 7, pp. 1201–1205.
19) Rashmi Sanghi and Preeti Verma 2009. A facile green extracellular biosynthesis of CdS nanoparticles by immobilized fungus, Chemical Engineering Journal 155, p886–

891.

20) Roy K., H.Q. Mao, S. Huang, K.W. KLeong 1999. Oral gene delivery with chitosan–DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. Nat. Med., 5 (), p. 387.
21) Sachlos, E.D. Gotora, J.T. Czernuszka 2006. Collagen scaffolds reinforced with biomimetic composite Nano-sized carbonated-substituted hydroxyapatite crystals and shaped by rapid prototyping to contain internal microchannels. Tissue Eng., 12 (), p. 2479.
22) Thoomatti S Anitha, Sudharsan and Palanivelu, Peramchi (2011) Synthesis and structural characterization of polydisperse silver and multi-shaped gold nanoparticles using Fusarium oxysporum mtcc 284. Digest Journal of Nanomaterials and Biostructures, 6 (4). pp. 1587-1595.
23) Vascashta, D. Dimova and Malinovska, 2005Nanostructured and nanoscale devices, sensors and detectors. Sci. Technol. Adv. Mater. 6, 312.
24) Vahabi, K., Mansoori, G. and Sedighe, K. (2011). Biosynthesis of Silver Nanoparticles by fungus Trichoolermareesei. In sciences Journal. 1(1), 65-79.
25) Waren C.W., S. Nie 1998. Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science, 281, p. 2016.
26) Zeinab S, Mojtaba S and Farzad K. 2011. Biological synthesis of gold nanoparticles by fungus Epicoccum nigrum, J Claus Sci 22. Pp 661-665.