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Research Article

BIOSYNTHESIS, PARTIAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF SILVER NANOPARTICLES FROM *PLEUROTUS* SPECIES

M. SHIVASHANKAR, B. PREMKUMARI AND N. CHANDAN
Department of Sericulture/Life Sciences, Bangalore University, Bangalore-560 056, Karnataka, India
Corresponding Authors email - shivashankarseri@gmail.com and premkumari1712@gmail.com

ABSTRACT

Integration of microorganisms to nanotechnology is one of the key issues in nanoscience research. There is growing need to develop environmentally benign metal nanoparticle synthesis process that do not use toxic chemicals in the synthesis protocols to avoid adverse effects in medical applications. The use of microorganisms in the synthesis of nanoparticles emerges as an eco-friendly approach. In the present study, biosynthesis of silver nanoparticles using edible mushrooms Viz., *Pleurotus pulmonarius, Pleurotus djamor* and *Hypsizygus (pleurotus)ulmarius,* partial characterization of silver nanoparticle and its antimicrobial study have been reported. It was found that the aqueous silver ions of 1-5mM concentration were reduced to silver metal nanoparticles by nitrate dependent reeducate and a shuttle quinone extracellular process when treated with fungal supernatant of *Pleurotus pulmonarius*, in 24hrs(2mM); *Pleurotus djamor* in 48hrs(5mM) and *Hypsizygus ulmarius* in 48hrs(3mM). Partial characterization of synthesized silver nanoparticles, investigated by UV-VIS Spectroscopy showed increased productivity at 386nm with sharp and intense surface plasmon. It is found that silver nanoparticles are bound to protein through carboxylate group of aminoacid residues. The synthesized silver nanoparticle showed high bactericidal activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria . The mechanism of the Ag NP bactericidal activity is discussed in terms of Ag NP interaction with the cell membranes of bacteria.

KEY WORDS: Silver nanoparticles, *Pleurotus pulmonarius, Pleurotus djamor Hypsizygus(pleurotus)ulmarius*, antimicrobial activity, extracellular synthesis.

INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. The synthesis of silver nanomaterials/nanoparticles is extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum period.

The microorganism probably play a role in providing a multitude of nucleation centers and establish conditions for obtaining high disperse nanoparticles system. They slow down or entirely prevent agitation by immobilizing the particle and providing a viscous medium (Sun *et al.,* 2002)

Among various metal nanoparticles, biologically synthesized silver nanoparticles has many applications that includes catalysts in chemical reactions (Kumar *et al.*, 2003), biolabelling, antimicrobial agent, electrical batteries (Peto *et al.*, 2002), drug and gene delivery (Rao *et al.*, 2001), DNA sequencing (Tripathi, 2003), staining pigments in glasses and ceramics and optical receptors (Klaus-Joerger, 2001, Krolikowska, 2003). It is known that, a large number of organisms, both unicellular and multi cellular are able to produce inorganic nanoparticles either intracellular or extracellular.

The use of fungi in the synthesis of nanoparticles is relatively a recent addition to the list of microorganisms. The use of fungi is potentially exciting since they secrete large amount of enzymes and their biomass are easy to handle. Using this unique property of fungi, it may be used to grow nanoparticles of silver as reported by Mukherjee *et al*., 2001b; Sastry *et al.,* 2003; Lloyd, 2003; Bhainsa *et al.,* 2006 and Vigneshwaran *et al.,* 2006.

Mushrooms are known to have anti-inflammatory, cardiovascular, antitumor, antiviral, antibacterial, hepatoprotective and hypotensive activities in biological systems (Wasser *et al.,*1999; Rai *et al.,* 2005; Bernardshaw, 2005). This indicates that, mushrooms could be valuable sources of antioxidant

(Chen *et al.*, 2006) and antitumor compounds (Cho *et al.*, 2003; White *et al.*, 2002). Studies on edible mushrooms have revealed valuable activities related to biological response modifications. Chemopreventive, chemotherapeutic, immunomodulatory, hypoglycemic and hypocholestemic effects (Lee *et al.*, 2006). Mushrooms characteristically contain many different bioactive compounds with diverse biological activity, the content and bioactivity of these compounds depend on how the mushroom is prepared and consumed (Chang 1996).

In the present investigation, biosynthesis, partial characterization of silver nanoparticles by using edible mushrooms viz.,*Pleurotus pulmonarius*, *Pleurotus djamor* and *Hypsizygu (pleurotus)ulmarius* and their antimicrobial activities was studied.

MATERIALS AND METHODS

All the chemicals used were of analytical grade. The media components like glucose, malt extract powder, malt agar, Mueller Hinton agar, silver nitrate were obtained from Hi-Media chemicals, Mumbai (India).

Source of fungal mycelia and culture maintenance

The white rot fungal mycelia of *Pleurotus pulmonarius*,(Plate1&2a,2b) *Pleurotus djamor* (Plate3&4a,4b) and *Hypsizygus ulmarius* (Plate5&6a,6b) were obtained from Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh. The mycelia were maintained at 4^0C on malt agar slants. Fungal filtrate used for biosynthesis, experiments were grown aerobically in liquid media containing 5g/l malt extract powder and 10g/l glucose. The fungal strain was inoculated in the autoclaved media under sterilized and static conditions and was allowed to grow for 120 hrs at 25^0C (150 rpm) with pH of 6.0 as reported by Rashmisanhi *et al.*, 2009.

Biosynthesis of silver nanoparticle

The cell free filtrate was obtained by filtration of *Pleurotus pulmonarius*, *Pleurotus djamor* and

Hypsizygus ulmarius using Whatmann.No.I filter paper. For the synthesis of silver nanoparticles 20ml of the cell free filtrate was brought in contact with different milimolar concentration in 150 ml Erlen Meyer flask and agitated at 25^0C in dark conditions under normal pH. Simultaneously control without silver ions was also run along with the experimental flasks (Nithya and Raghunathan, 2009).

Characterization of Silver Nanoparticles

UV-VIS studies

The reduction of silver ions was monitored by UV-VIS spectrum at 24hrs, 48hrs and 72hrs time interval by drawing 1cm^3 of the sample. The absorbance was recorded at a resolution of 0.5nm at 350-800nm using UV-VIS spectrophotometer (Elico, UV-VIS SL 191).

Bacterial susceptibility to nanosilver

Susceptibility of the synthesised silver nanoparticles was done by well diffusion method as reported by Nithya and Raghunathan 2009, Nelson Duran *et al*., 2007 on Mueller Hinton agar plates with Gram positive- *Staphylococcus aureus* and gram negative- *Pseudomonas aeroginosa* organisms. The zone of inhibition was calculated for its antimicrobial studies.

RESULTS

Formation of nanosilver

Pleurotus pulmonarius depicts the color change from pale yellow to mild light brown at 2mM for 24hrs with further decreases in intensity of color at different mM concentrations of silver nitrate solutions. (Plates7a and 7b).

PLATE-7a

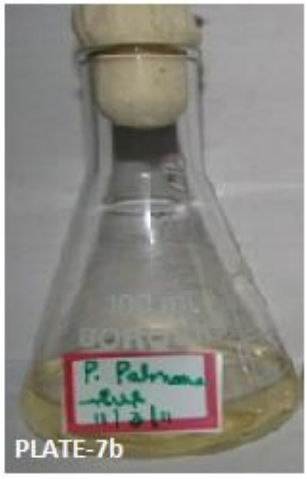


PLATE-7b

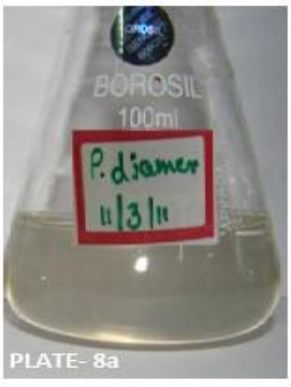


PLATE- 8a

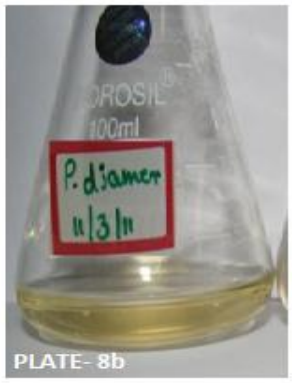


PLATE- 8b

Pleurotus djamor shows the color change from pale yellow to light pinch brown at 5mM for 48hrs that further decreased in intensity of color with different mM concentrations of silver nitrate solutions. (Plates 8a and 8b)

Hypsizygus ulmarius when observed shows the color change from pale yellow to pale brown at 3mM for 48hrs with maximum intensity when compared to different mM concentrations of silver nitrate solutions with decrease in intensity of color. (Plates 9a and 9b).

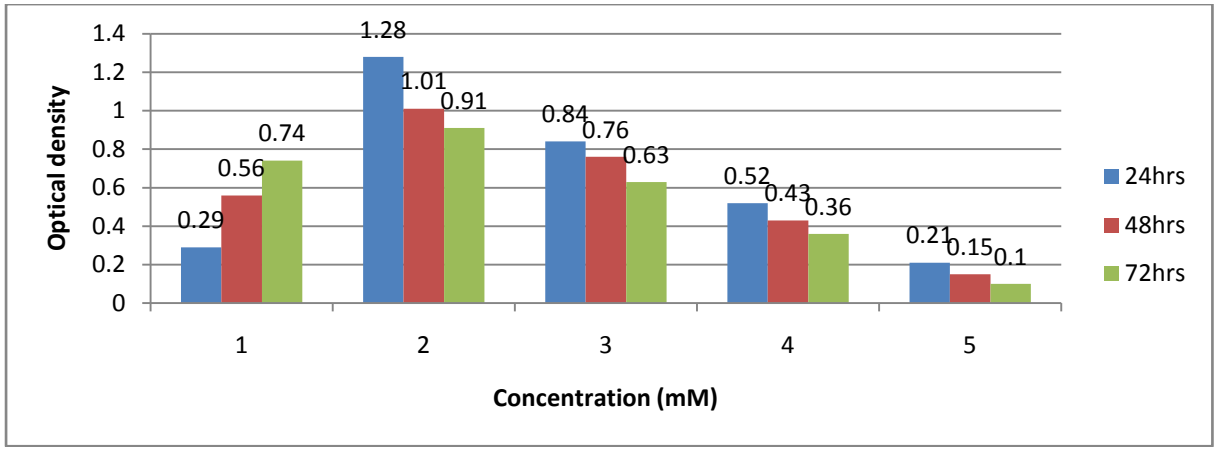
PLATE- 9a

PLATE-9b

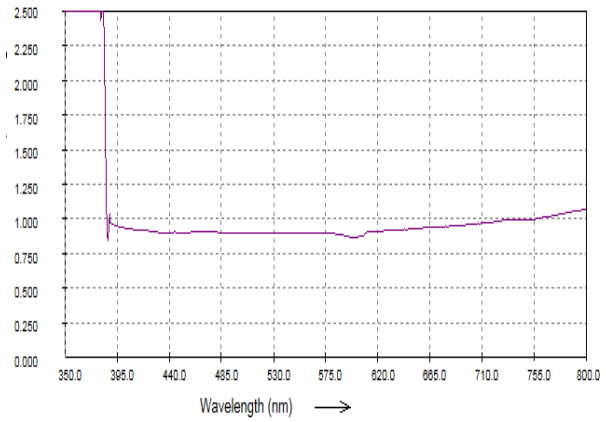
UV-VIS spectral analysis

UV–visible spectra of different fungal supernatent treated with 1-5 different mM concentration of silver nitrate solutions showed a characteristic surface Plasmon absorption band at 386 nm. Further incubation, lead to decrease or increase in intensity indicating complete or maximum reduction of silver ions.

Characteristic surface plasmon absorption band at 386 nm was observed at 2mM concentration for 24hrs (Graph 1) with optical density of 1.28 (Graph 2) for *Pleurotus pulmonarius* (Table 1); *Pleurotus djamor* showed at 5mM concentration for 48hrs (Graph 3) with optical density of 0.98 (Graph 4 and Table 2) and at 3mM concentration for 48hrs (Graph 5) with optical density of 1.222(Graph 6) for *Hypsizygus ulmarius* (Table 3)



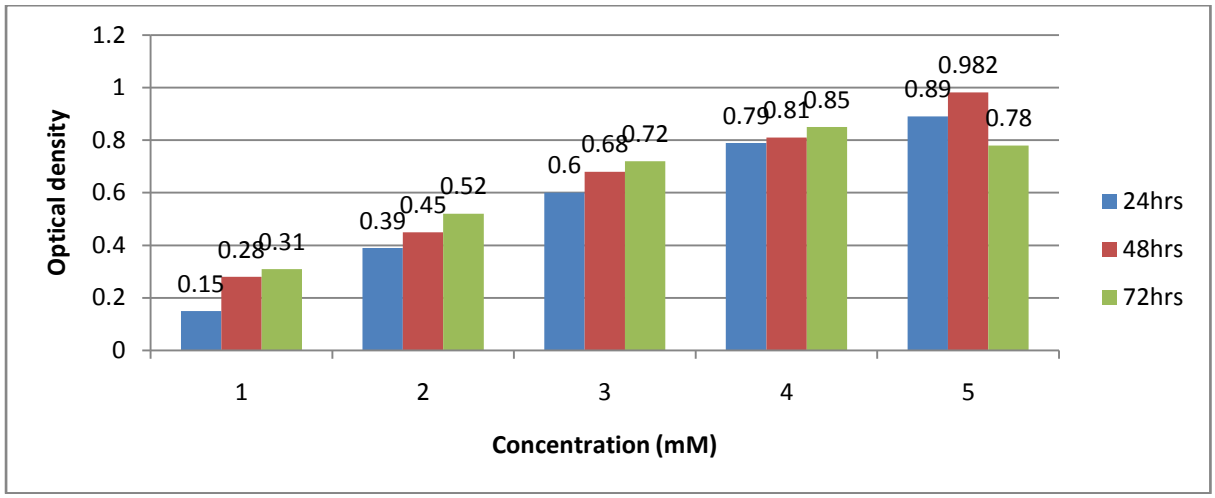
Graph 1: Depicts record of silver nanoparticles synthesised by *Pleurotus pulmonarius* at various time Intervals against optical density (Y- axis) and sample concentration (X- axis).



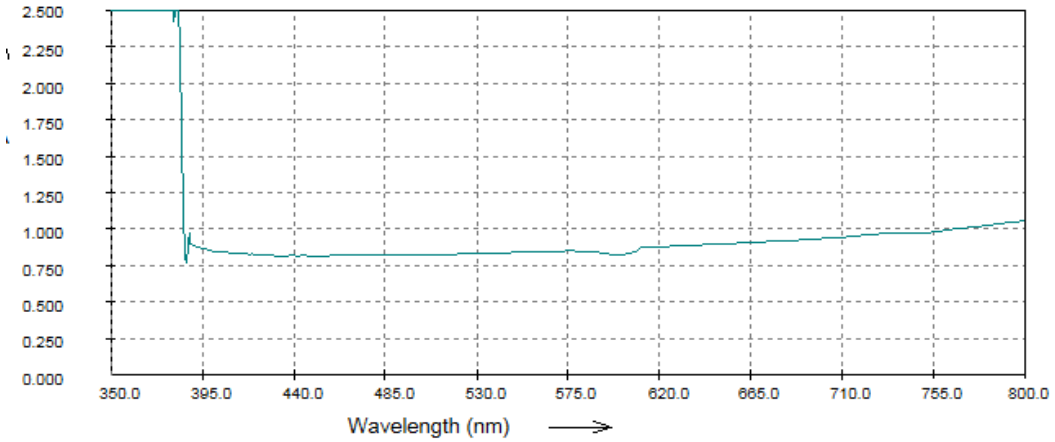
Graph 2: Transverse surface Plasmon absorbance band at 386nm of *Pleurotus pulmonarius* with absorbance at (Y-axis) and wavelength (X-axis)

Concentration (mM)	24hrs	48hrs	72hrs
1	0.29	0.56	0.74
2	**1.28**	1.01	0.91
3	0.84	0.76	0.63
4	0.52	0.43	0.36
5	0.21	0.15	0.10

Table 1: O.D values of *Pleurotus pulmonarius*in different mM concentration at different time intervals.



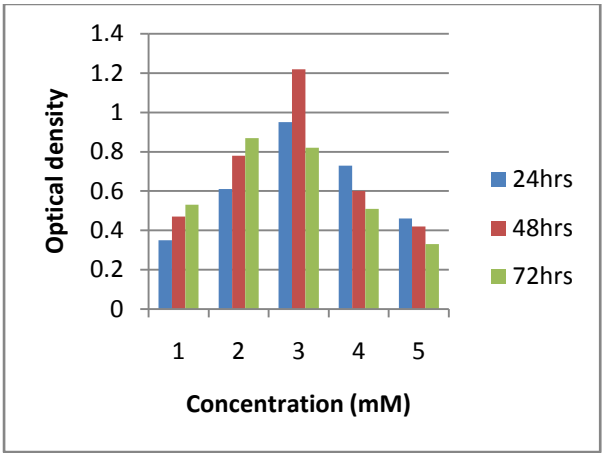
Graph 3: Depicts record of silver nanoparticles synthesized by *Pleurotus djamor* at various time intervals against optical density (Y- axis) and sample concentration (X- axis)



Graph 4: Transverse surface Plasmon absorbance band at 386nm of *Pleurotus djamor* with absorbance (Y-axis) and wavelength (X-axis)

Concentration (mM)	24hrs	48hrs	72hrs
1	0.15	0.28	0.31
2	0.39	**0.45**	0.52
3	0.60	0.68	0.72
4	0.79	0.81	0.85
5	0.89	**0.98**	0.78

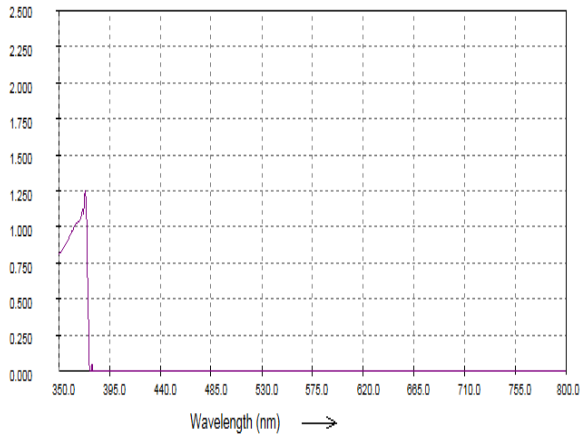
Table 2: O.D values *Pleurotus djamor* in different mM concentration at different time intervals.



Graph 5: Depicts record of silver nanoparticles synthesised by *Hypsizygus ulmarius* at various time Intervals against optical density (Y- axis) and sample concentration (X- axis).

Concentration (mM)	24hrs	48hrs	72hrs
1	**0.35**	**0.47**	**0.53**
2	**0.61**	**0.78**	**0.87**
3	**0.95**	**1.22**	**0.82**
4	**0.73**	**0.60**	**0.51**
5	**0.46**	**0.42**	**0.33**

Table 3: O.D values *Hypsizygus ulmarius* in different mM concentration at different time intervals.



Graph 6: Transverse surface Plasmon absorbance band at 386nm of *Hypsizygus ulmarius* with absorbance (Y-axis) and wavelength (X-axis)

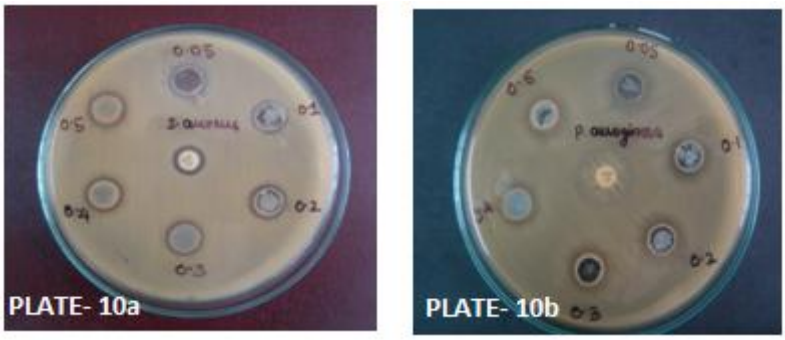
Bacterial susceptibility to synthesized nanosilver

The antibacterial activity of synthesised silver nanoparticles of *Pleurotus pulmonarius* ,*Pleurotus djamor* and *Hypsizygus ulmarius* on gram positive and gram negative organisms are recorded with clear zone of inhibition in centimeter, with ampicillin10mcg/disc as control.

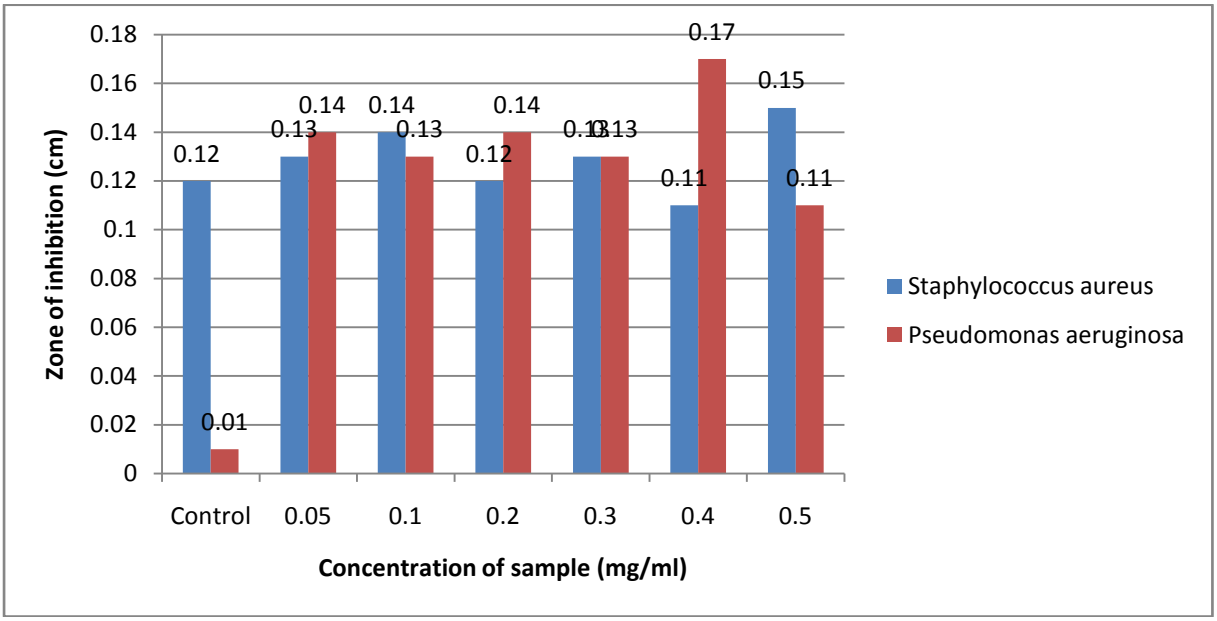
Pleurotus pulmonarius showed inhibition zone (Plates10a and 10b) of 0.15cm in *Staphylococcus aureus* at 0.5mg/ml and 0.17cm in *Pseudomonas aeruginosa* at 0.4mg/ml along with control 10mcg/disc with inhibition diameter of 0.12cm and 0.01cm. (Graph 7, Table 4).

In *Pleurotus djamor* maximum inhibition zone (Plates 11a,11b) was seen in *Staphylococcus aureus* at a concentration 0.4mg/ml along with control 10mcg/disc with inhibition diameter of 0.14cm and 0.12cm whereas *Pseudomonas aeruginosa* recorded at a concentration of 0.2mg/ml along with control 10mcg/disc with inhibition diameter of 0.19cm and 0.02cm (Graph 8, Table 5).

Hypsizygus ulmarius showed inhibition zone (Plates12a,12b) of 0.16cm in *Staphylococcus aureus* at 0.3mg/ml and 0.16cm in *Pseudomonas aeruginosa* at 0.05mg/ml along with control 10mcg/disc with inhibition diameter of 0.12cm and 0.03cm. (Graph 9, Table 6).



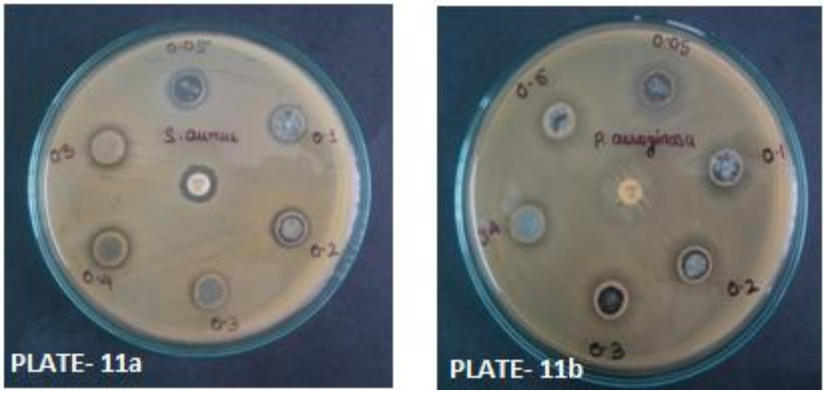
Culture plate showing the Antibacterial activity of synthesized silver nanoparticles by *Pleurotus pulmonarius* against *Staphylococcus aureus* (10a) and *Pseudomonas aeroginosa* (10b)



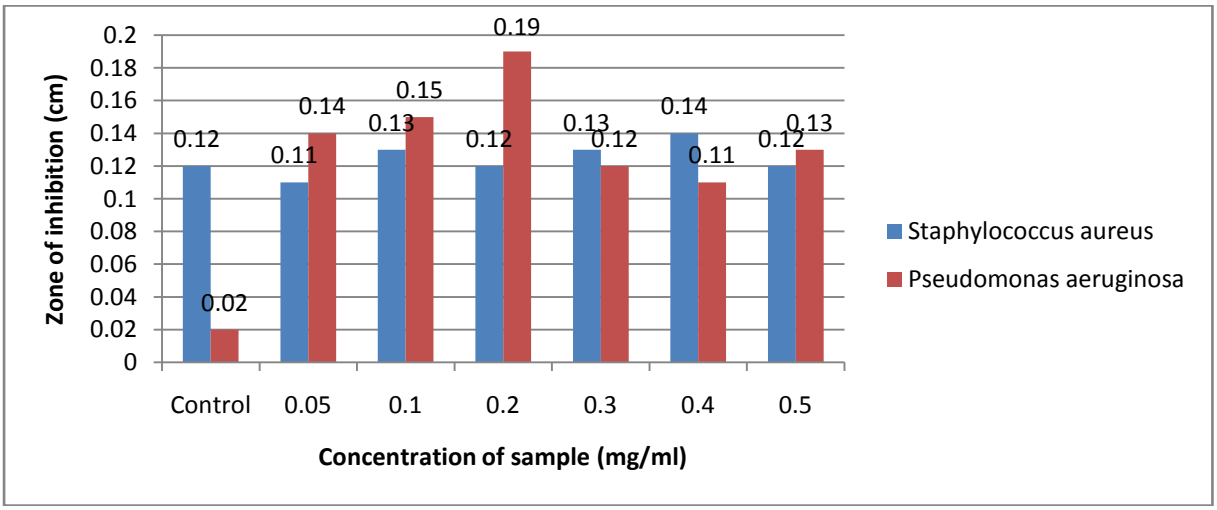
Graph7: Antibacterial activity of synthesized silver nanoparticles by *Pleurotus pulmonarius*(mg/ml) representing zone of inhibition(cm) on *S. aureus* & *P. aeroginosa*

Concentration of sample mg/ml	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
	Zone of inhibition (cm)	
Control	0.12	0.01
0.05	0.13	0.14
0.1	0.14	0.13
0.2	0.12	0.14
0.3	0.13	0.13
0.4	0.11	**0.17**
0.5	**0.15**	0.11

Table 4: Record of inhibition zone (cm) by synthesized silver nannoparticles of *Pleurotus pulmonarius* at different concentration with respect to *S.aureus* and *P.aeruginosa*



Culture plate showing the Antibacterial activity of synthesized silver nanoparticle by *Pleurotus djamor* against *Staphylococcus aureus* (11a) and *Pseudomonas aeroginosa* (11b)

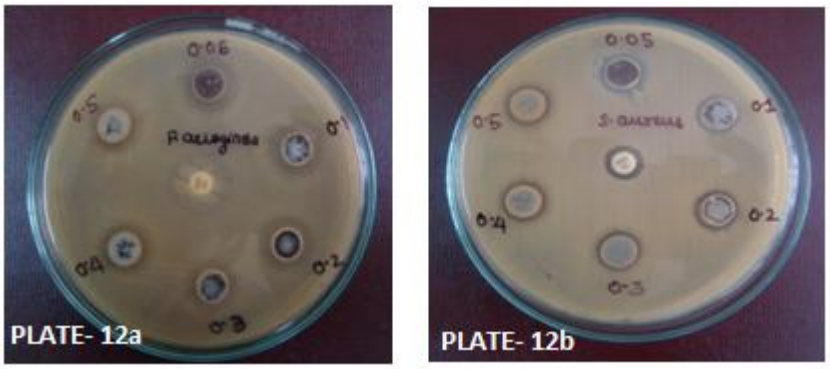


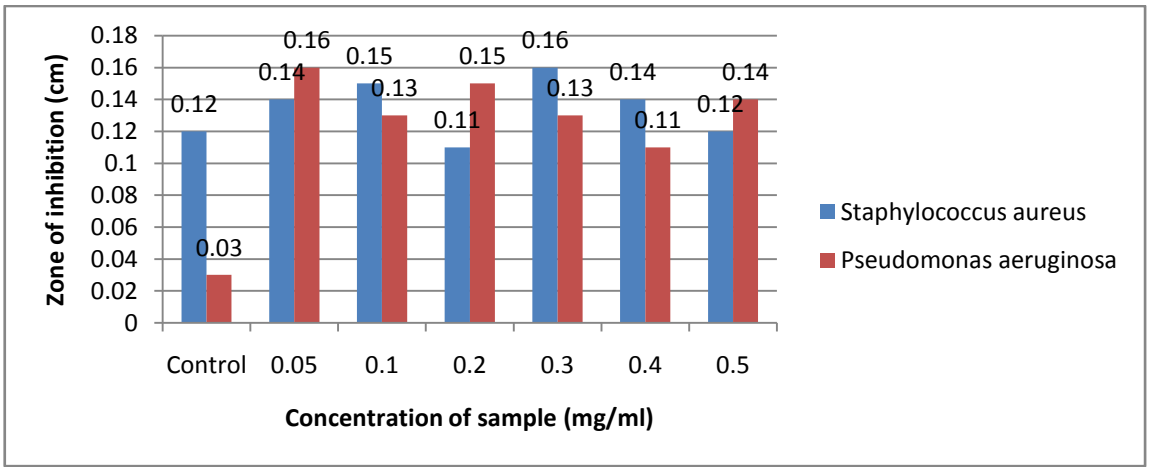
Graph 8: Antibacterial activity of synthesized silver particles by *Pleurotus djamor* (mg/ml) representing zone of inhibition (cm) on *S. aureus* & *P. aeroginosa*

Concentration of sample mg/ml	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
	Zone of inhibition (cm)	
Control	0.12	0.02
0.05	0.11	0.14
0.1	0.13	0.15
0.2	0.12	**0.19**
0.3	0.13	0.12
0.4	**0.14**	0.11
0.5	0.12	0.13

Table 5: Record of inhibition zone (cm) of synthesized silver nannoparticles by *Pleurotus djamor* at different concentration with respect to *S.aureus* and *P.aeruginosa*

Culture plate showing the Antibacterial activity of synthesized silver nanoparticle by *Hypsizygu sulmarius* against *Staphylococcus aureus* (12a) and *Pseudomonas aeroginosa* (12b)





Graph 9: Antibacterial activity of synthesized silver particles by *Hypsizygu sulmarius* (mg/ml) representing zone of inhibition (cm) on *S. aureus* & *P. aeroginosa*

Concentration of sample mg/ml	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
	Zone of inhibition (cm)	
Control	0.12	0.03
0.05	0.14	**0.16**
0.1	0.15	0.13
0.2	0.11	0.15
0.3	**0.16**	0.13
0.4	0.14	0.11
0.5	0.12	0.14

Table 6: Record of inhibition zone (cm) of synthesized silver nannoparticles by *Hypsizyg usulmarius* at different concentration with respect to *S.aureus* and *P.aeruginosa*

DISCUSSION

Nanotechnology is an existing area of scientific development and is a field of convergence among life sciences, material science and information technology. It is an emerging field of science capable of resolving issues and problems that are impossible to tackle in engineering and biological sciences.

Accumulation of metals by biological species may be metabolism-independent "adsorption" (biosorption) at the cell surface or metabolism-dependent internal "absorption" to organelles, cytoplasmic ligands and cytoplasmic structures (Gadd,1988). Extracellular production of silver nanoparticles by the filtered culture supernatants with aqueous silver nitrate solution, from 1-5mM for 24, 48 and 72hrs exist, produced the maximum synthesis of silver nanoparticles at 2mM concentration with in 24hrs for *Pleurotus pulmonarius*, 5mM concentration, 48hrs for *Pleurotus djamor* and at 3mM concentration, 48hrs for *Hypsizygus ulmarius*. The appearance of light brown colour clearly indicates the formation of silver nanoparticles in the reaction mixture along with the control. Sadowski *et al.*, 2008; Nikhil *et al*., 2009; Maliszewska *et al*., 2009 and Kannannatrajan *et al*., 2010 reported that upon addition of silver ions into the filtered cell free filtrate in the dark, samples changes its color from almost colourless to brown with intensity increasing during the period of incubation. Kowshik *et al.,* 2003 reported the conversion of 3mM silver nitrate solution to nanosilver by *Fusarium oxysporum* in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown. The characteristics brown colour of colloidal silver solution is due to the excitation of surface plasmon vibrations in the nanoparticle providing a convenient spectroscopic signature of their formation. Several hydroquinones with excellent redox properties were reported that could be act as electron shuttle in metal reductions as reported by Baker *et al.,* 1998 ;Newman *et al.,* 2000. Thus, it was evident that, electron shuttle or others reducing agents released by *Pleurotus pulmonarius, Pleurotus djamor, Hypsizygus ulmarius* are capable of reducing silver ions to silver nanoparticles. On the other hand, the reduction of silver ions did not occur in the absence of fungal cells. It indicates that the reducing agents that are released into the cultures of *Pleurotus pulmonarius, Pleurotus djamor, Hypsizygus ulmarius* are involved in the reduction process.

UV-VIS spectrum is one of the important and easy technique to verify the formation of metal nanoparticles provided surface plasmon resonance exists for the metal. UV–visible spectra of fungal supernatant treated with the silver nitrate solutions showed a maximum characteristic surface plasmon absorption band at 386 nm for *Pleurotus pulmonarius* at 2mM,24hrs with OD 128 when compared to *Pleurotus djamor* at 5mM, 48hrs with OD 1.98 and *Hypsizygus ulmarius at* 3mM 48hrs with OD 1.22 . Further incubation lead to decrease in intensity indicating complete reduction of silver ions.

According to Kowshik *et al.*, 2003, the absorption at 280nm indicated the presence of tryptophan, tyrosine or phenylalanine residues in the protein, indicating the release of proteins into filtrate that suggests a possible mechanism for the reduction of metal ions present in the solution.

Observation of the strong but broad surface plasmon peak has been well known in the case of various metal nanoparticles over a wide size range of 2-100 nm by Kowshik *et al.*, 2003. Shankar *et al.*, 2003 suggested that the shoulder at 370-390nm corresponded to the Transverse plasmon vibration in silver nanoparticles, whereas the peak at 440nm due to excitation of longitudinal plasmon vibrations.

Various forms of silver ions and nanoparticles are widely studied in the biological systems and have reported to have strong inhibitory and antimicrobial effects against many fungal and bacterial pathogens causing economically important diseases in plants (Morons *et al.*, 2005; pal *et al.*, 2007; kim *et al.*, 2008; 2009, kabir *et al.*, 2011). Many patents are filed for nano silver for preservation and treatment of diseases in agricultural field (Anderson, 2009).
Silver is now and expected agrochemical replacement, which eliminates unwanted microorganisms in soils. Silver is an excellent plant growth stimulator (Morons *et al.*, 2005; kim *et al.*, 2008; 2009, kabir *et al.*, 2011).

Silver nanoparticles were evaluated for use in increasing the antimicrobial activities of different antibiotic agents S. aureus and E.coli. the antibacterial activities of pencillin G, amoxicillin, erythromycin, clindamycin and vancomycin increased in the

presence of Ag-Np's against the test strains. Shanmugam *et al.,* 2006 reported that silver nanoparticles inhibit the growth of *E. coli, S. aureus, salmonella* species, *pseudomonas* species, *bacillus* species, and *K. Pnemoniae,* indicating broad spectrum of antimicrobial activity. Among Me-Np's silver nanoparticles have been known to have inhibitory and bactericidal effect (*cho et al.,* 2005).

In the present study, the synthesized silver nanoparticle solution of *Hypsizygus ulmarius* at concentration of 0.3mg/ml and 0.05mg/ml exhibited excellent antibacterial activity against the bacteria *Staphylococcus aureus* (0.16cm) and *Pseudomonas aeruginosa* (0.16cm) when compared to *Pleurotus pulmonarius* at a concentration of (0.05mg/ml), with 0.15cm inhibition zone for *Staphylococcus aureus,* 0.4mg/ml with 0.17cm for *Pseudomonas aeruginosa* and 0.4mg/ml, with 0.14cm inhibition zone for *Staphylococcus aureus*,0.2mg/ml, 0.19cm for *Pseudomonas aeruginosa.*

The extent of inhibition of bacterial growth reported in this study was dependent on the concentration of nanoparticles in the muller-hinton medium. Interaction between nanoparticles and the cell wall of bacteria would be facilitated by relative abundance of negative charges on the Gram negative bacteria.

Reports on the inhibitory action of silver ions on microorganism's shows that upon silver ion treatment DNA loses its replication ability (Feng *et al.,* 2005) and expression of ribosomal subunit proteins as well as cellular proteins and enzymes essential to ATP production becomes inactivated. Mritunjai singh *et al.,* 2008 reported that antibacterial effect was size and dose dependent and was more pronounced against Gram negative bacteria than Gram positive bacteria.

CONCLUSION

Microbes have been reported to reduce metal ions and stabilize nanoparticles with a wide size range (Chen *et al.,* 2003; Vigneshwaran *et al.,* 2007). In this study, three fungus *Pleurotus pulmonarius, Pleurotus djamor, Hypsizygus ulmarius* was used for the synthesis of stable silver nanoparticles which was quite fast, efficient, ecofriendly and formed within hours when silver ions came in contact with cell filtrate. The UV-VIS Spectra showed characteristic surface plasmon absorption band at 386nm. These silver nanoparticles showed excellent antibacterial activity against two representative pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Since the fungus of *Pleurotus pulmonarius, Pleurotus djamor, Hypsizygus ulmarius* is commercially accepted edible mushroom, the byproduct after harvesting the mushroom is available in abundance for the large scale production of silver nanoparticles. The use of agricultural bio mass in nanotechnology will result in the growth of multidisciplinary research activities. Further development of eco-friendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology

REFERENCES

1. *Baker RA, Tatum JH: 1998. Novel anthraquinones from stationary cultures of Fusariumoxysporum. J Ferment Bioeng.* ***85.*** *359-361*
2. *Bernardshaw E, Johnson G, Hetland, Scand J. 2005. An extract of the mushroom agaricusblazeimurill administered orally protects against systemic streptococcus pneumoniae infection in mice. Immun.****62****. 393-398.*
3. *Bhainsa KC, D'Souza SF. 2006. Extracellular biosynthesis of silvernanoparticles using the fungus Aspergillusfumigatus. Colloids Surf BBiointerfaces.* ***47****. 160-164.*
4. *Brause R, Barbic M, Smith DR, Schultz DA, Schultz S. 2002. Localized surface plasmon resonance of single silver nanoparticles studied by dark-field optical microscopy and spectroscopy. J.Chem.Phys.****116****. 6755.*
5. *Chang R. 1996. Functional properties of edible mushrooms. Nutr Rev.* ***54****. S91-S93.*
6. *Chen S, Oh SR, Phung S, Hur G, Ye JJ, Kwok SL, Shrode GE, Belury M, Adams SL, Williams D. 2006. Anti-aromatase activity of phytochemicals in white button mushrooms (Agaricusbisporus). Cancer Res.****66****. 12026.*
7. *Cho J, Kang JS, long PH, Jing J, Back Y, Chung KS. 2003. Antioxidant and memory enhancing effects of purple sweet potato anthocyanin and cordyceps mushroom extract. Arch. pharm. res.* ***26****. 821-825.*
8. *Feng QL, Wa J, Chen GQ, Cui KZ, Kim TM, Kim JO. 2005. A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. J. Biomed Mater.Res. 662-668.*
9. *Gadd GM. & Rome L. de 1988. Biosorption of copper by fungal melanin. Appi. Microbial. Biotechnol.,****29.*** *6 1 0-6 17.*
10. *KannanNatarajan, SubbalaxmiSelvaraj,V. RamachandraMurty. 2010. Microbial production of silver nanoparticles.Digest Journal of Nanomaterials and Biostructures.****5.*** *135 – 140.*
11. *Klaus-Joerger T, Joerger R, Olsson E, and Granqvist CG. 2001. Bacteria as workers in the living factory: metal-accumulating bacteria and their potential for materials science. Trends in Biotechnology.* ***19****.15–20.*
12. *Kowshik, ShriwasAshtaputre, Sharmin Kharrazi. 2003. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3. Nanotechnology.****14****. 95–100.*
13. *Krolikowska A, Kudelski A, Michota A, Bukowska J. 2003. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. Surf Sci.* ***532****. 227-232.*
14. *Kumar A, Mandal S, Selvakannan PR, Parischa R, Mandale AB, Sastry M. 2003. Investigation into the interaction between surface-bound alkylamines and gold nanoparticles. Langmuir.* ***19****.6277–6282.*

15. *Lee JS, Lim MO, Cho KY, Cho JH, Chang SY, NamDH. 2006. Identification of medicinal mushroom species based on nuclear large subunit rDNA sequences. J. Microbiol.* ***44****. 29-34.*
16. *Lloyd JR.2003. Microbial reduction of metals and radionuclides.FEMS Microbiol Rev****. 27****. 411-425*
17. *Maliszewskaand M. Puzio. 2009. Extracellular Biosynthesis and Antimicrobial Activity of Silver Nanoparticles.Actaphysicapolonica .****116.***
18. *Mrittunjaisingh, Shinjinisingh S, Prasada, I & S Gambhir. 2008. Nanotechnology in Medicine and Antibacterial effect of silver nanoparticles. Digest journal of nanomaterials and biostructures. 115-122.*
19. *Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parischa R, Ajayakumar PV, Alam M, Kumar R, Sastry M. 2001. Fungus mediatedsynthesis of silver nanoparticles and their immobilizationin the mycelial matrix: A novel biological approach to nanoparticle synthesis. Nano Lett,* ***1****.515-519.*
20. *Nelson Durán, Priscyla D, Marcato, Gabriel IH, De Souza, Oswaldo L Alves, and Elisa Esposito. 2007. Antibacterial Effect of Silver Nanoparticles Produced by Fungal Process on Textile Fabrics and Their Effluent Treatment. Journal of Biomedical Nanotechnology.****3****. 203–208.*
21. *Newman DK, Kolter R. 2000. A role for excreted quinones in extracellular electron transfer. Nature.* ***405.****94-97.*
22. *Nikhil S. Shaligram , Mahesh Bule , Rahul Bhambure , Rekha S. Singhal ,Sudheer Kumar Singh , George Szakacs , Ashok Pandey. 2009. Biosynthesis of silver nanoparticles using aqueous extract from the compactin producing fungal strain. Process Biochemistry* ***44****. 939–943.*
23. *Nithya R, Raghunathan R. 2009. Synthesis of silver nanoparticle using Pleurotussajorcaju and its Antimicrobial study. Digest journal of nanomaterials and biostructures.4. 623-629.*
24. *Peto G, Molnar GL, Paszti Z, Geszti O, Beck A, Guczi L. 2002. Electronic structure of gold nanoparticles deposited on SiO_x/Si(100). Materials Science and Engineering.****19****. 95-99.*
25. *Rai M, Tidke G,* ***Wasser SP.*** *2005. Therapeutic potential of mushrooms. Natural Products Radiance.* ***1****. 246-257*
26. *Rao CNR, Cheetham AK. 2001. Science and technology of nanomaterials: current status and future prospects. J. Mater. Chem.* ***11****. 2887*
27. *Rashmisanghi, Preetiverma. 2009. Biomimetic synthesis and characterisation of protein capped silver nanoparticles.Bio res.techol. 501-504.*
28. *Sadowski Z, Maliszewska IH, Grochowalska B, Polowczyk I, Kozlecki T. 2008.Synthesis of silver nanoparticles using microorganisms. Material science .****26****:419-424*
29. *Sastry M, Ahmad A, Islam Khan M, Kumar R. 2003. Biosynthesis of metal nanoparticles using fungi and actinomycetes. Current Science.* ***85****(2):162–170.*
30. *Shankar SS, Ahmad A, Parischa R, Sastry M. 2003. Bioreduction of chloroaurate ions by geranium leaves and its endophytic fungus yields gold nanoparticles of different shapes. J Mater Chem. 1822–1826.*
31. *Sun Y, Xia Y. 2002. Shape-controlled synthesis of gold and silver nanoparticles.Science.* ***298****:2176–9.*
32. *Tripathi GNR. 2003. p-Benzosemiquinone radical anion on silver nanoparticles in water.J. Am. Chem. Soc.****125****, 1178-1179.*
33. *Vighneshwaan N, Kathe AA, Vardadarajan PV, Nachane RP, Bala Subramanian RH. 2007. Silver-Protein (Core-Shell) Nanoparticle Production Using Spent Mushroom Substrate. Langmuir.****23****. 7113-7117*
34. *Vigneshwaran N, Kathe AA, Varadarajan PV, Nachane PR, Balasubramanya RH. 2006. Biomimetics of silver nanoparticles by white rot fungus, Phaenerochaetechrysosporium. Colloids Surf B.****53****.55–59.*
35. *Wasser SP, Weis AL. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspective (review). Int. J. Med. Mushrooms.****1****. 31–62.*
36. *White RW, Hackman RM, Soares SE, Beckett LA, Sun B. 2002. Effects of a mushroom mycelium extract on the treatment of prostate cancer. Urology.* ***60****. 640-644.*
37. *Shanmugam S, Viswanathan B, Varadarajan TK. 2006. A novel single step chemical route for noble metal nanoparticles embedded organic-inorganic composite films. Mat. Chem. Phy.* ***95.****51-55*
38. *Pal S, Tak YK, Song JM. 2007. Does the antimicrobial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram negative bacterium Escherichia coli. Appl. Environ. Microbiol.****73.*** *1712-1720.*
39. *Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee DG. 2008. Antifungal effect of silver nanoparticles on dermatophytes. Journal of microbiology and biotechnology.****18****. 1482- 1484.*
40. *Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG. Lee DG. 2009. Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals,* ***22.*** *235-242.*
41. *Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramı´rez JT, Yacaman MJ. 2005. The bactericidal effect of silver nanoparticles. Nanotechnology.* ***16.*** *2346–2353.*
42. *Cho K, Park J, Osaka T, Park S. 2005. The study of antimicrobial activity and preservative effects of nanosilver ingredient. Electrochim Acta.* ***51.*** *956-960.*
43. *Syed Rashel Kabir, Md. Azizul Haque, Md. Nurujjaman, Imtiaj Hasan, Md. Abu Zubair, Md. Robiul Hassan Chowdhury, Md. Belal Uddin, Narayan Roy, Md. Kamrul Islam, Nurul Absar. 2011. Study of antimicrobial activities of chitinases from a potato prototype cultivated in Bangladesh. Malaysian Journal of Microbiology.* ***7(2).*** *92-96*
44. *Anderson Cal Baier. 2009. Regulating nanosilver as a pesticide", Environmental Defense Fund, February 12.*