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Biosynthesis of Silver Nano Particles from *Fusarium oxysporum* Culture

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ABSTRACT

From a very long time inhibitory effect of silver has been recognized and used towards many bacterial strains and microorganisms commonly present in medical and industrial processes. The most useful and important characteristic of silver is its antimicrobial property. In the current study silver nano particles with uniform size distribution of 10-20nm with stability and promising increase in yield were obtained and effect of cultural and physical conditions on biological synthesis of SNPs was studied. SNPs (Silver Nanoparticles) synthesis was first analysed out by visual observation of colour change of the fungal filtrate after treatment with silver nitrate ($AgNO_3$) solution within 24hrs. Formation of dark brown colour of fungal cell filtrate indicated the presence of SNPs (Silver Nanoparticles).

INTRODUCTION

According to most taxonomists fungi have worldwide distribution having around 100,000 species but the global biodiversity of the fungus kingdom is still not fully understood. Most fungi grow by forming hyphae, as a vegetative body which are thread –like, cylindrical structures 2-10µm and up to several centimetres in length. *Fusarium* is one of the largest genuses of these filamentous fungi. It is widely distributed in soil, water, plants, etc. Fusarium mycelia can be visible to the naked eye. They secrete a large amount of protein. Currently Nanoparticles research becomes an area of scientific interest due to its various applications in different fields like biomedical, optical and electronic areas(Taylor et al.,2013; Hewakuruppu et al., 2013). Nanoparticles are available in different sizes ranging from coarse particles (2,500 and 10,000 nm), fine particles (100 and 2,500 nm), and ultrafine particles (1 and 100 nm) in size.The term "Myconanotechnology" was coined for the synthesis of Nanoparticles by using fungi(Rai et al., 2009).

Copper, zinc, titanium, magnesium, gold, alginate, and silver are the different types of metals which are being used for synthesis of nanomaterials . But the biological synthesis of silver nanoparticles by using filamentous fungus of Fusarium species and the characterization of these synthesized silver nanoparticles with the help of UV- visible Spectroscopy is also possible after doing intensive research on Fusarium sp. Nanoparticles application and its uses in various fields includes molecules in research and diagnosis of diseases, creating fluorescent biological labels for important biological markers, gene delivery systems for gene therapy and drug delivery systems, for estimation of proteins , isolation and purification of biological molecules and cells in research, probing of DNA structure, genetic and tissue engineering, tumours destruction with drugs or heat, studies in MRI, in pharmacokinetic studies and other areas(Jain,2008;Kayser *et al*., 2005; Pathak and Thassu ,2009). Uses of fungus *Fusarium* for the production of extracellular metal nanoparticles is one of the easiest ways for the production of relatively quick and ecologically clean metallic nanoparticles. Synthesis of silver nanoparticles (SNPs) by these fungi is an emerging technology and can be treated as an important branch of nanotechnology due to its eco-friendly, safe, and cost effective nature.

From ancient time silver were used either in the form of metallic silver, silver nitrate, or silver sulfadiazine. The uses of silver have been again realized in the form of silver nanoparticles (SNPs), which can be synthesized from different fungal strain. These particles showed significant antimicrobial activity against the multidrug resistant microorganisms owing to their reduced size (Savithramma et al., 2011).The extensive uses of Silver Nanoparticles in medicine and researches demands the easy, quick and eco-friendly procedure for its synthesis. Researchers at MIT are using nanoparticles to deliver vaccine. The vaccine is protected by nanoparticles, allowing the vaccine time to trigger a stronger immune response. Scientists are developing different ways to use carbon nanodiamonds in medical applications like testing the uses of chemotherapy drugs attached to nanodiamonds for the treatment of brain tumors, leukemia, etc. Silver Nanoparticles are especially used in biosensors and numerous assays as biological tags for quantitative detection.

MATERIALS & METHODS

Collection of waste Banana peel

The waste banana peel was collected from the different part of Greater Noida. Then the isolated Banana peel was dissolved into 100ml Distilled water with 0.85gm NaCl and purified with serial dilution and isolates were identified on the basis of culture characteristics and microscopic characteristics similar to *Fusarium oxysporum*. After mixing streaking were done on to PDA (Potato Dextrose Agar) plates and Plates were incubated at 28^{0}C for 4-5days (Fig.1).

Isolation and morphological identification of fungus

The test fungus was isolated from decayed banana peel on potato dextrose agar and incubated at 280c for 4-5 days. Fungal individual colonies were then picked up and further purified by sub-culturing on potato dextrose agar media (Fig.2). The fungus was identified by cultural (texture of mycelia, colony colour) and microscopic characteristics (macro and micro-conidia and chlamydospores).

Microscopic characterization

Smear was made and stained by Lectophenol cotton Blue. Then the slides were examined microscopically under 40X. Morphology of the cell was recorded with relation to morphology of spore chain, mycelium structure and hyphae (Fig.3).

Synthesis of Silver nanoparticles (extracellular)

The mycelia of *Fusarium* were inoculated in 200ml Erlenmeyer flasks, each containing 250ml of PDB (Potato Dextrose Broth) medium and kept flasks on rotary shaker for 5 days. Later mycelia were harvested by filtration through whatman filter paper no. 42. After this process 10g biomass was immersed in 100ml of distil water for 72h, at 28^{0}C and then the aqueous component was separated by filtration. Into this solution 2ml of 1mM $AgNO_3$ was added and an overall Ag+ concentration was obtained in a solution of 10^{-3}M.

Silver Nanoparticles characterization

SNPs (Silver Nanoparticles) detection was carried out by colour change of the fungal filtrate after treatment with silver nitrate ($AgNO_{3)}$ with in 24hrs. Dark brown color appearance of fungal cell filtrate indicated the formation of SNPs (Silver Nanoparticles).

UV-Visible Spectroscopic Analysis

Silver Nanoparticles (SNPs) were characterized using UV-visible dual beam spectrophotometer by analyzing the absorbance spectra in range of 200-800nm of wavelength. To study the influence of the reaction time, we measured UV-visible spectra of the reacting sample at regular time intervals of 5min to 45min. Initially the colour of the filtrate was pale

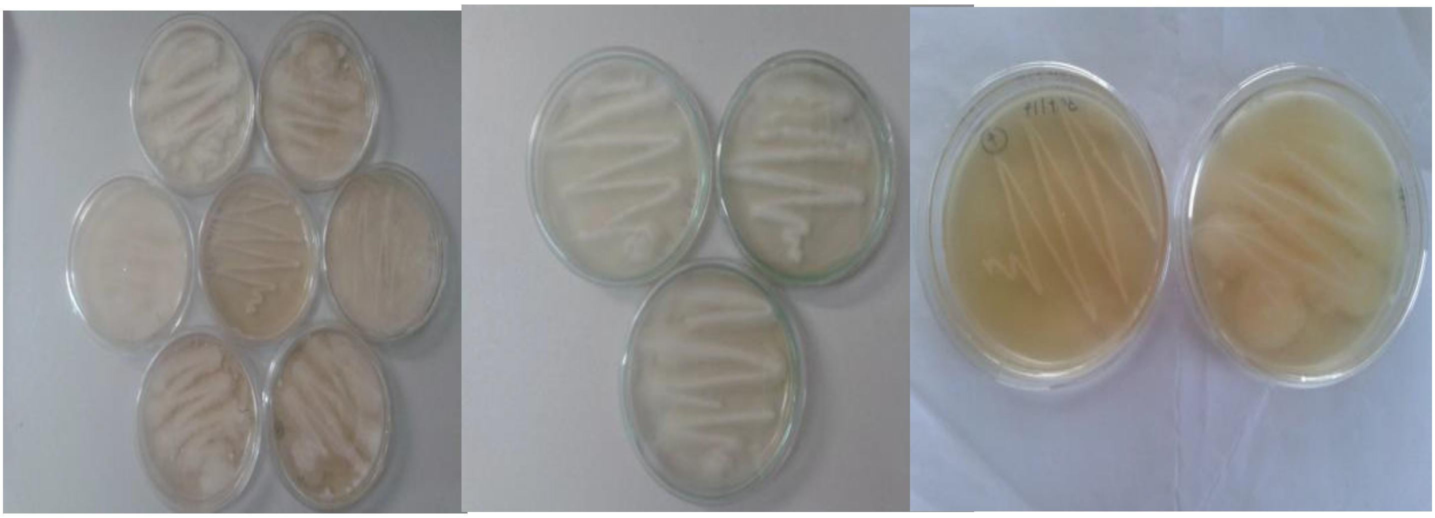


Fig.1 Growth of Banana cells on PDA (Potato Dextrose Agar) Plates.

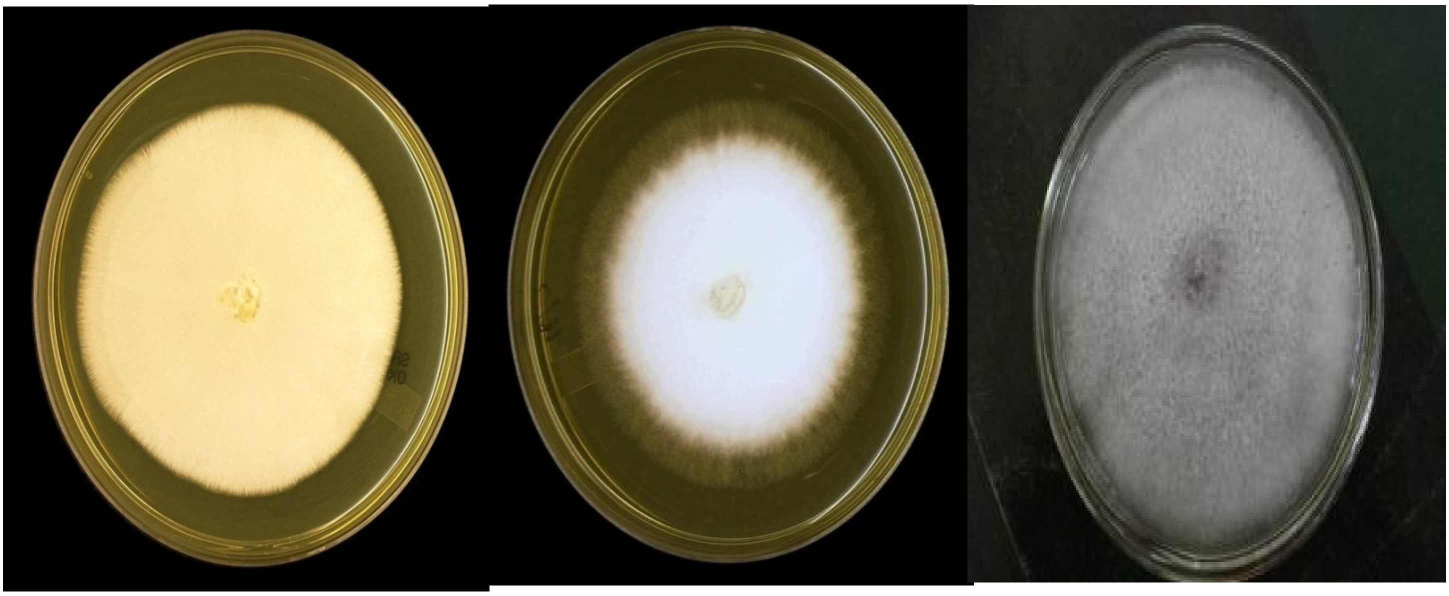


Fig.2 Colony Morphology of the Fusarium species isolates.

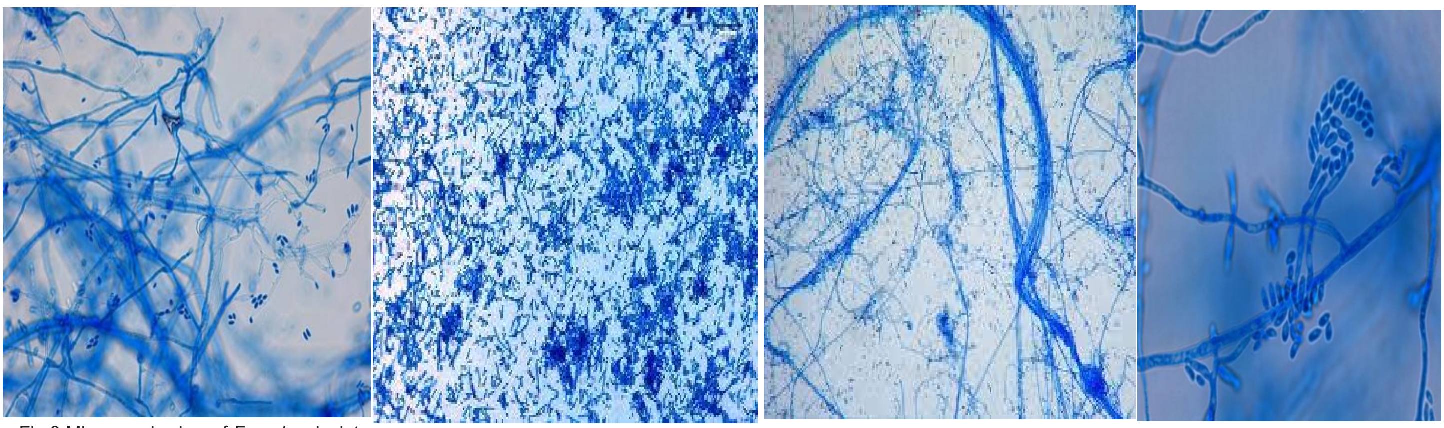


Fig.3.Microscopic view of *Fusarium* isolates

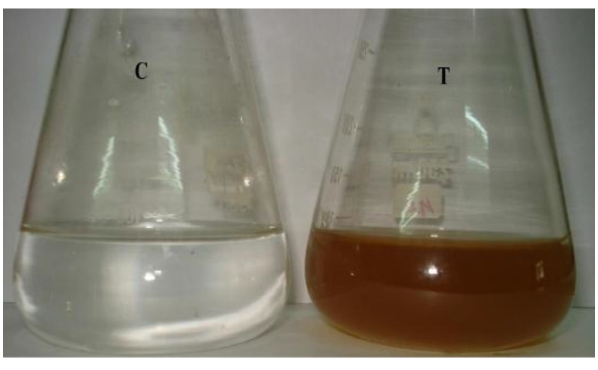
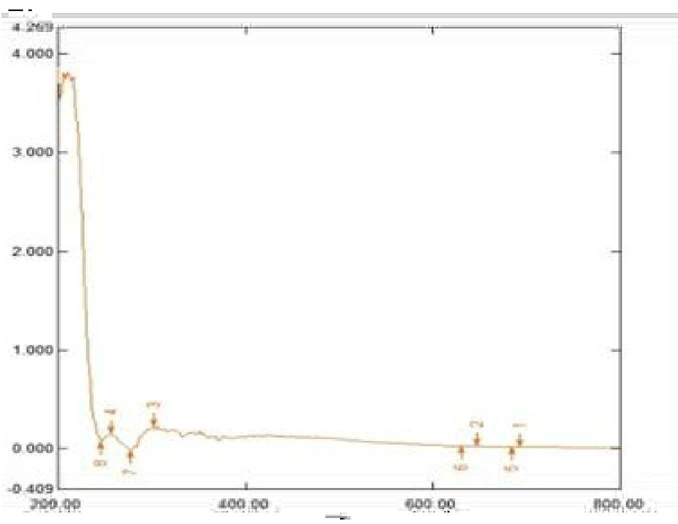


Fig.4 Biosynthesis of Silver Nanoparticles –colour change reaction: conical flasks containing the extracellular filtrate of *Fusarium* sp.biomass (C) and conical flask containing the extracellular filtrate of the Fusarium sp. Biomass after exposure to AgNO3 solution for 24 hr. (T).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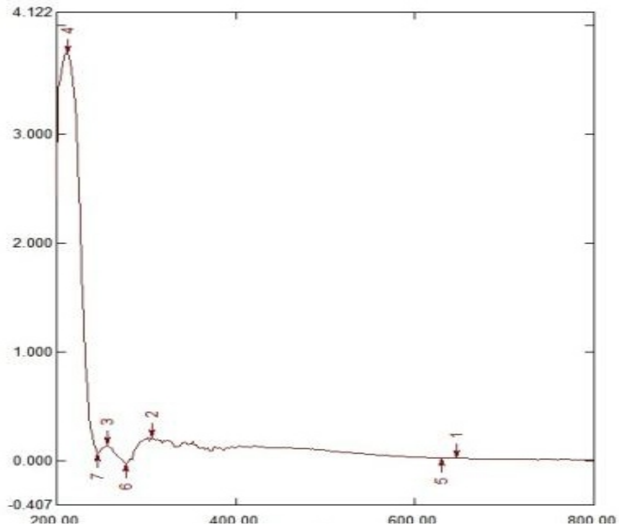
600.00
800.00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1.000
0.000
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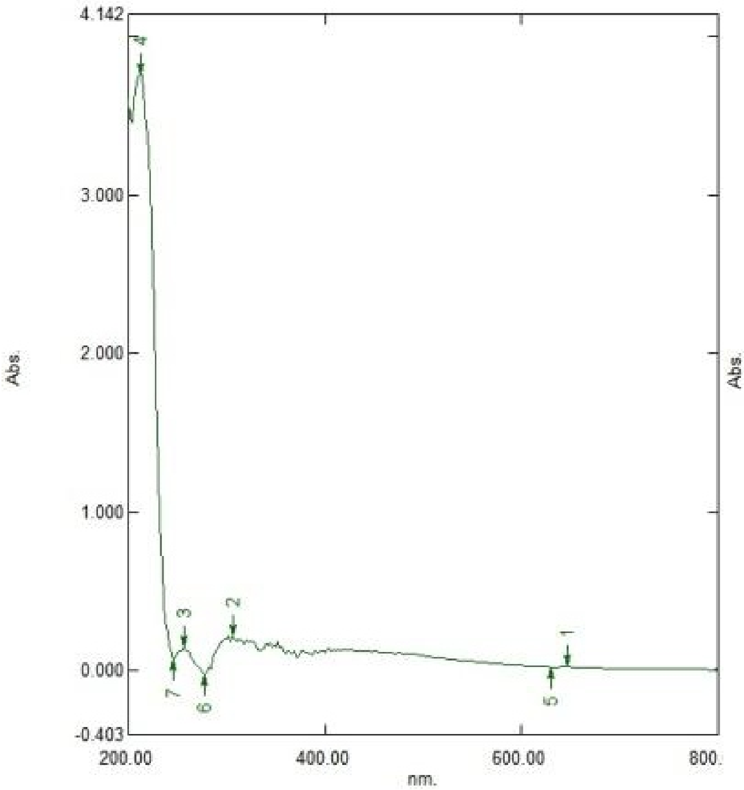
600.00
800.00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200.00
400.00
600.00
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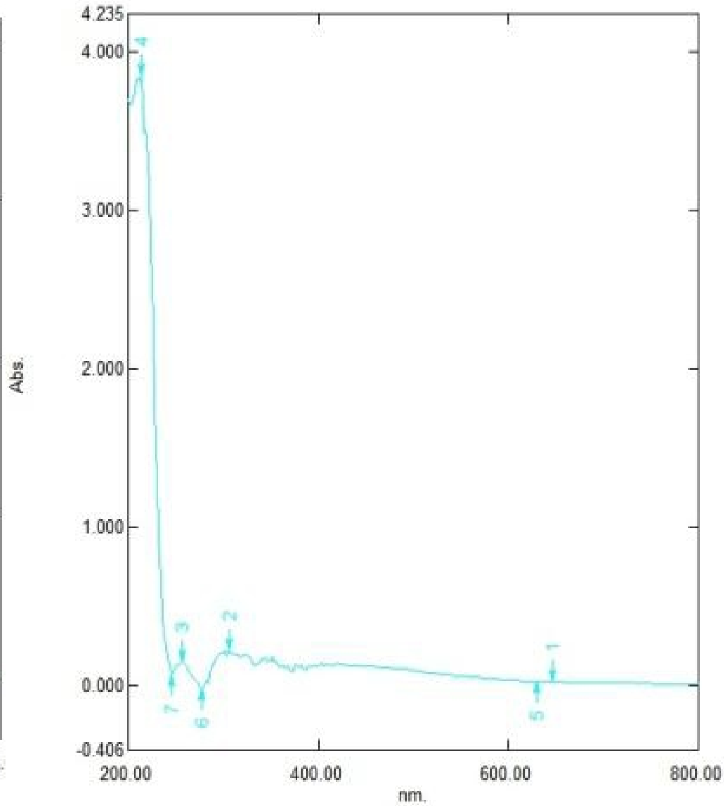
nm.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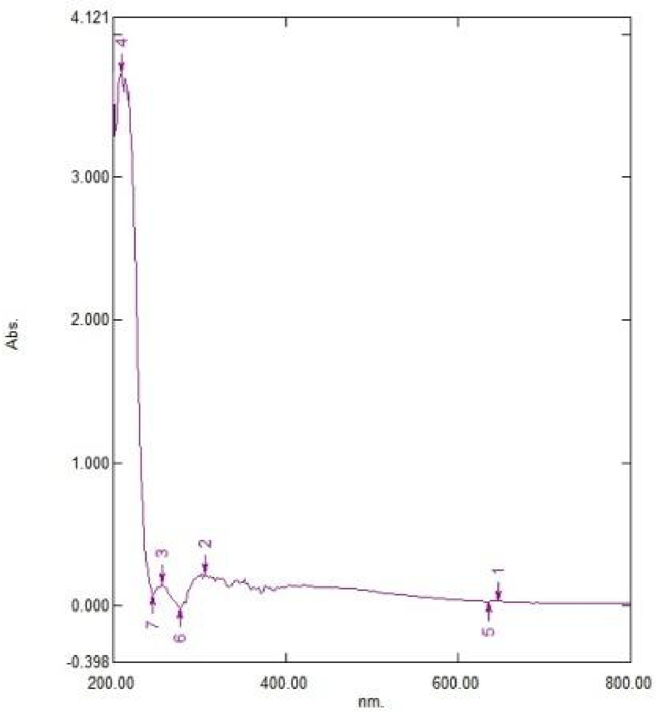
800.00
nm.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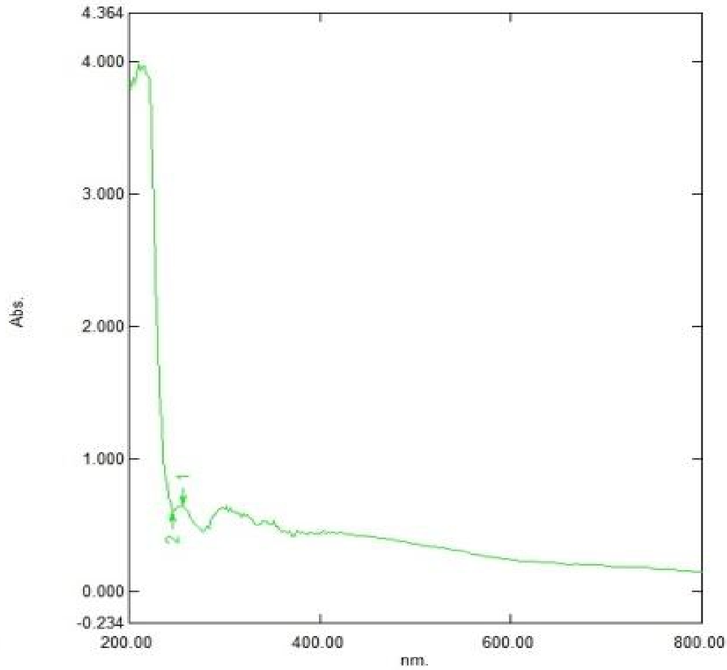
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600.00
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nm.

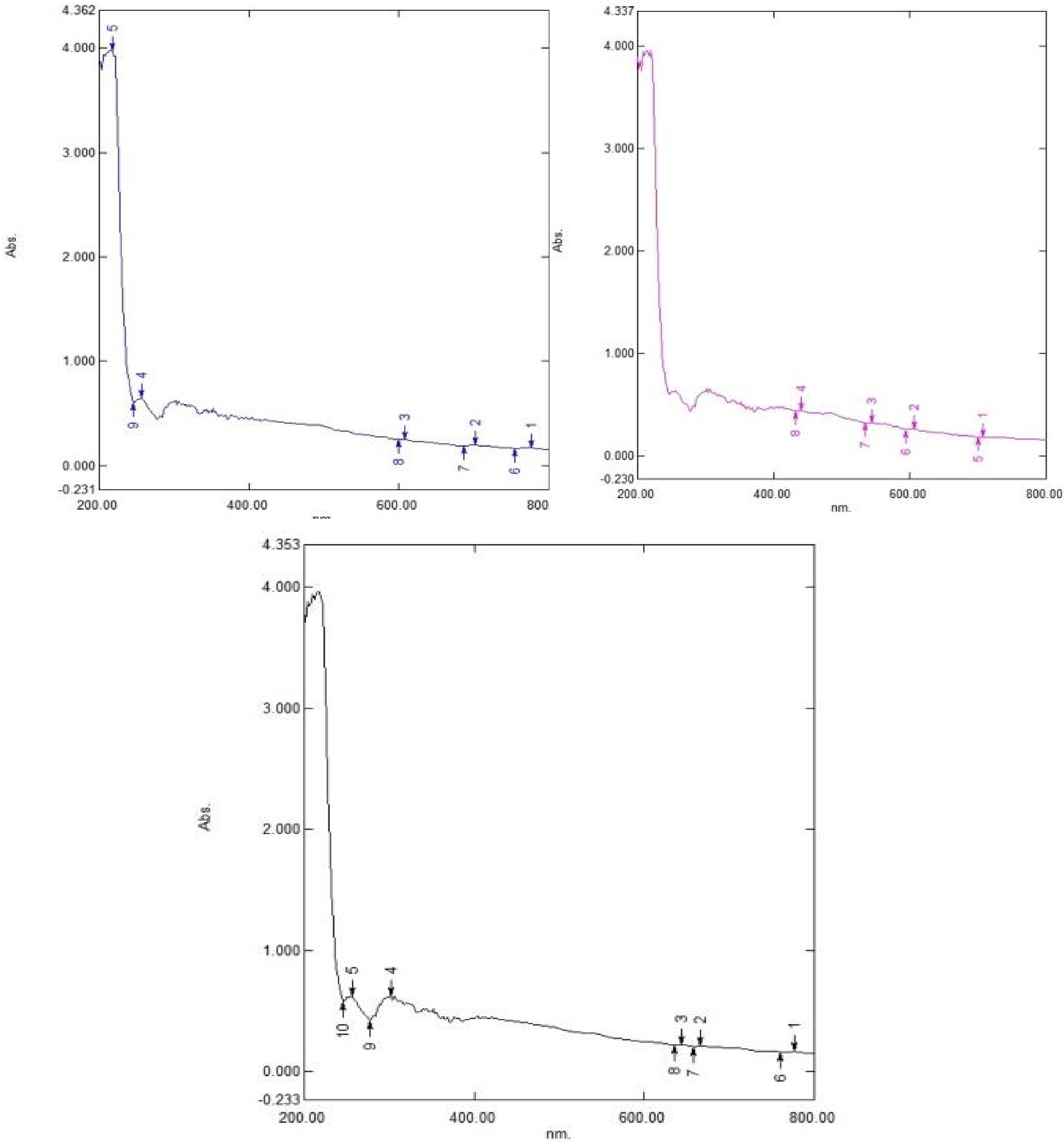

Fig.5 UV-visible spectrum of silver Nanoparticles synthesized using Fusarium sp. after 24 hr.

yellow, and as time passes colour changes to brown followed by dark-brown with increase in the absorbance, which indicates the continuous synthesis of Silver Nanoparticles (SNPs) in filtrate .

RESULT AND DISCUSSION

In various fields metallic nanoparticles are of interest for its applications ranging from acting as catalysts and optics sensing, data storage and antibacterial activity because it shows different properties when exhibiting on its different sizes and shapes (Vilchis-Nestor et al., 2008;Yoosaf et al., 2007). Silver Nanoparticles when incorporated in footwear, paints, wound dressings, appliances, and even in cosmetics, and plastics shows their antibacterial properties. Because of these antibacterial properties they are now-days are extensively used in food packaging, clothing, disinfectants and household appliances. Metallic nanoparticles are synthesized traditionally by using wet chemical methods but in today's time use of chemicals could increase the cost as well and also it pollutes the environment. This is why the green technology is in demand. An anamorphic species which is *Fusarium oxysporum* was identified by morphological methods and shared by both pathogenic and non-pathogenic strains. *Fusarium* is found to be most diverse in population in nature. In our laboratory banana peel extract was used and from this silver nanoparticles were synthesised. (Fig 1,2,3). Similarly Banker et al., (2010) also reported that peel extract of banana leads to a novel pathway which helped in the synthesis of silver nanoparticles. With the help of this non-toxic and environment-friendly biological material which is banana peel extract (BPE)), it was possible to develop a bio-inspired synthesis procedure. Shankar *et al.*, (2008)also performed the same experiments for the synthesis of Silver Nanoparticles with Geranium leaf. These Geranium leaf extract were made to treat with silver nitrate solution (aqueous). Synthesis of highly stable, crystalline silver nanoparticles in the solution was detected by rapid reduction of the silver ions. In our lab SNPs (Silver Nanoparticles) synthesis was detected by observing the change in colour of the fungal filtrate on reacting with silver nitrate ($AgNO_3$) solution within 24hrs. Appearance of dark brown colour in fungal cell filtrate indicated the formation of SNPs (Silver Nanoparticles) (Fig.4). The fungus was identified by cultural (texture of mycelia, colony colour) and microscopic characteristics (macro and micro-conidia and chlamydospores). We also identified it by using fungal Genomic DNA extraction kit. Through this kit we observed change in colour from colourless to pale yellow which is carried out by *Fusarium mycelium* that turns pink or purple with age. This study could also be used as an important advancement in the use of plants over microorganism in the biosynthesis of silver nanoparticles.

However, there is an increased issues related to the impacts of the use of silver nanoparticles on biological system in large scale and also its possible risks to the health and environment (Renata *et al*., 2012). Using dual beam UV-visible spectrophotometer, Silver Nanoparticles (SNPs) were also characterized by observing the spectra absorbance in range from 200-800nm wavelength. To study the influence of the reaction time, we measured UV-visible spectra of the reacting sample at regular time intervals of 5min to 45min. Initially the colour of the filtrate was pale yellow, and as time passes colour changes to brown followed by dark-brown with increase in the absorbance, which indicates the continuous synthesis of Silver Nanoparticles (SNPs) in filtrate (Fig.5). Nowadays, there is an increased commercial demand for nanoparticles due to their vast application in a number of areas such as chemistry, electronics catalysis, energy and medicine. Combining all optimized conditions, we have developed an eco friendly and inexpensive method for the rapid and large scale synthesis of SNPs from Fusarium sp. However, so far there is no report on the study of effect of all these cultural and physical conditions on biological synthesis of SNPs. Cultural (culture medium quantity of biomass, filtrate volume and salt concentration) and physical conditions (pH, temperature and light intensity) have been found to determine the affect of maximum yield, rate of synthesis and size of SNPs. The optimization of the parameters would lead to the rapid and large scale production of SNPs at industrial level, which may be used as novel antimicrobials against multidrug resistant microorganisms.

Several useful characteristics of Silver nanoparticles have made it the vastest applicable nanoparticles in science and the most important characteristic is related to its antimicrobial activity. Silver which has been long observed as the most useful nanoparticle due to its inhibitory property towards many microorganisms including bacterial strain commonly found in medical and industrial processes. This colour change observation of the medium which occurs very rapidly to brownish colour due to the excitation of surface Plasmon vibrations, typical of the silver nanoparticles. In this study, and due to our experience in optimization of biotransformation reactions, the reaction mixture and was successfully optimized to increase the yield of nanoparticles production.

CONCLUSION

On combining all optimized conditions, eco-friendly and inexpensive method has been developed for the rapid and large scale synthesis of SNPs. However, so far there is no report on the study of effect of all these cultural and physical conditions on biological synthesis of SNPs. Culture (culture medium quantity of biomass, filtrate volume and salt concentration) and physical conditions (pH, temperature and light intensity) have been found to affect the maximum yield, rate of synthesis and size of SNPs. In combination with all desired conditions, SNPs with uniform size distribution of 10-20nm with stability and promising increase in yield were obtained. The study revealed that the synthesis of SNPs may take place due to amino acids. The optimization of the parameters would lead to the rapid and large scale production of SNPs at industrial level, which may be used as novel antimicrobials against multidrug resistant microorganisms. The colour of the medium changed very rapidly to brownish due to the excitation of Plasmon vibrations on surface, typical of the silver nanoparticles. Due to our experience in optimization of biotransformation reactions and through this study we were able to be optimized our reaction mixture and increased the yield of nanoparticles production. Thus, the necessary information for economical production of silver nanoparticles by *Fusarium* was collected.

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