When in comparison with bacteria, mold can produce larger amounts of nanoparticles because they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles. The mechanism of silver nanoparticle production by mold is said to follow the following steps: trapping of Ag^+ ions at the surface of the mold cells and the subsequent reduction of the silver ions by the enzymes present in the mold system. The extracellular enzymes like naphthoquinones and anthraquinones are said to facilitate the reduction. Considering the example of *F. oxysporum*, it is believed that the NADPH-dependent nitrate reductase and a shuttle quinine extracellular process are responsible for nanoparticle formation. Though the exact mechanism involved in silver nanoparticle production by mold is not fully deciphered, it is believed that the abovementioned phenomenon is responsible for the process. A major drawback of using microbes to synthesize silver nanoparticles is that it is a very slow process when in comparison with plant extracts. Hence, the use of plant extracts to synthesize silver nanoparticles becomes an option that is feasible.

5.1 Strain Selected

Trichoderma reesei NCIM 992 microbial mold strain was acquired from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India.

Conversion of Silver Nitrate salt into Silver Nanoparticles

$$2AgNO_3 + 2e^- \rightarrow 2Ag^0 + O_2 + 2NO_2 \quad (6)$$

The Ag^+ **reduction**, by any **reducing agent** to Ag^0 takes place by a **redox reaction**. This includes the liberations of O_2 and NO_2 that is described by Equations 6. NO_2 and O_2 is released.

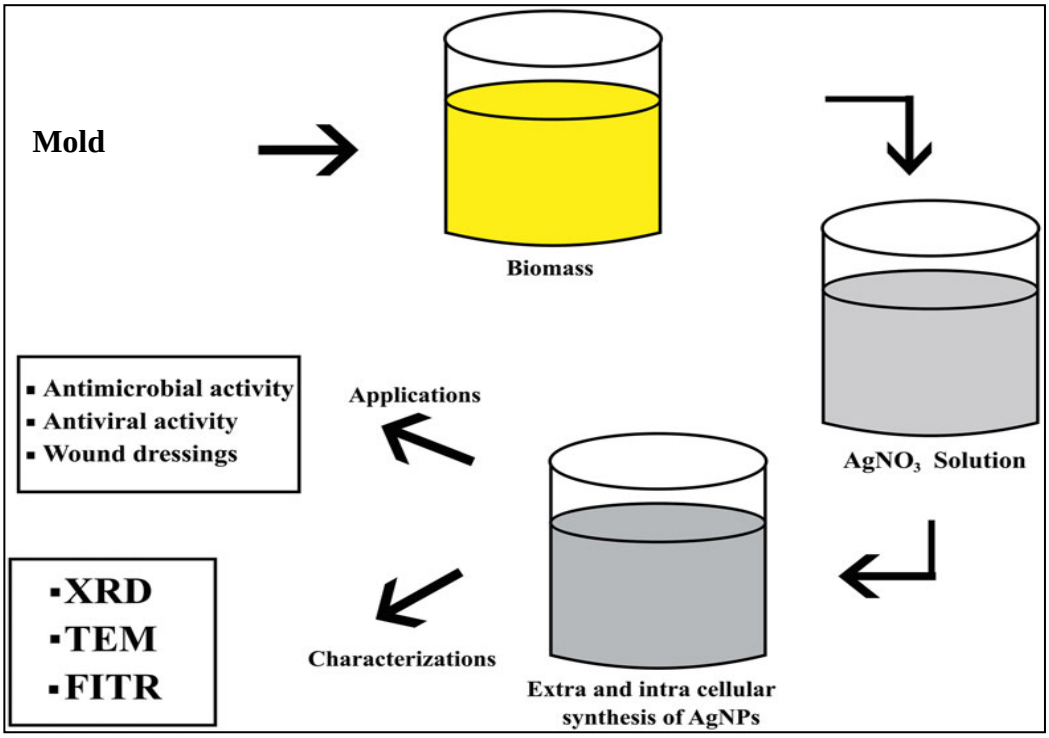

Figure 5.1: Brief description of Silver Nanoparticles

5.2 Growth characteristic comparison of control and experimental media (with Silver Nitrate) for *Trichoderma reesei* NCIM 992

Cell pellets from each of the identical flasks was emptied at regular time intervals and packed cell volume (PCV) was measured. It is used for the study of growth pattern of mold.

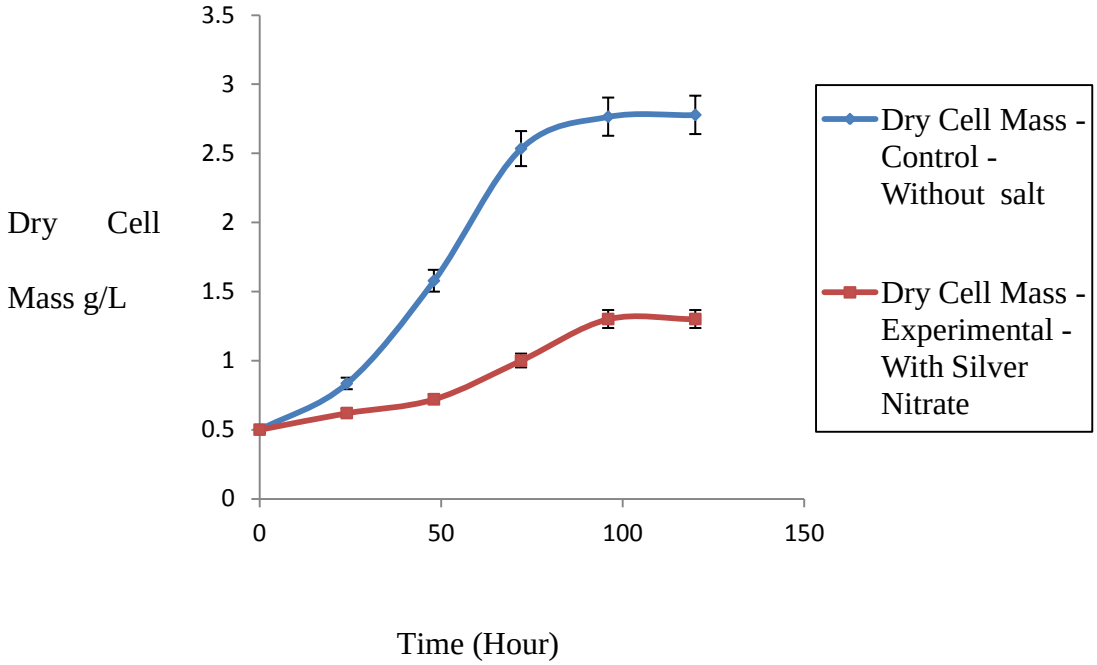

Fig. 5.2: Comparative growth pattern of two different *Trichoderma reesei* NCIM 992 strains i.e. without and with Silver Nitrate solution

Growth pattern of the various mold strains were studied both in normal nutrient media as well as in the presence of silver nitrate to analyze the effect of salt on the growth of mold strains. In the presence of silver nitrate, there was decrease in growth, as compared to normal growth pattern. It was due to inhibitory effect of silver nitrate on the mold growth. The limited growth is associated with the reduction of the salt. Due to this, the mold biomass media turned into brown in colour. Growth associated nanoparticle reduction is not very efficient as shown. Therefore, it is economical and beneficial to grow the biomass first and then incubating in gold chloride.

5.3 Formation of Silver Nanoparticles by using *Trichoderma reesei* NCIM 992

Under aseptic conditions, sufficient cell mass of *Trichoderma reesei* was separated and washed thrice with distilled water. 20 grams of wet biomass was taken and suspended in to a 500 ml Erlenmeyer flask containing 100 ml of 1 mM $AgNO_3$ solution. Above culture was incubated at 28°C at 200 rpm for 2 days. For control, biomass was suspended in distilled water and incubated at 28°C at 200 rpm for two days.

The amount of Silver Nanoparticles produced was measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS of the residual Gold Chloride salt revealed that more than 75% of the Gold Chloride salt was converted to nanoparticles as shown in **Table 5.1**.

Table 5.1: ICP-MS of the Residual Silver Nitrate Salt

Metal Salt	**Original Salt Concentration**	**Residual Salt Concentration**	**Percentage of Bioreduction of Silver Nitrate Salt**
Silver Nitrate Salt	30 ppm	6.1	79.5

5.4 Characterization of Silver Nanoparticles

5.4.1 UV-Visible Spectral Studies of Silver Nanoparticles

UV-Vis. Spectroscopy study of the lightly sonicate samples for the Surface Plasmon Resonance showed peaks which is based on the particle sizes of Silver Nanoparticles. As the particle size of Silver Nanoparticles varies between 5 – 100 nm, the value of peak wavelength changes between 393 – 462 nm, which is characteristic of Silver Nanoparticle. Similarly, the absorbance of Silver Nanoparticle solution measured at a wavelength of Surface Plasmon peak and its intensity varied with the mold strains, indicating the possible differences in Silver Nanoparticle size and morphology.

The UV-Vis. Spectra of blank Czapek Dox Broth media supplemented with Silver Chloride did not shows any contribution (Figure 5.4). The Surface Plasmon peak for Silver Nanoparticles produced by *Trichoderma reesei* NCIM 992 was recorded as 450 nm (Figure 5.5). The spectral shift accompanied by broadening of the Surface Plasmon Resonance could be attributed to the increment in gold nanoparticle size [Kumar *et al.* (2008), Njoki *et al.* 2007].

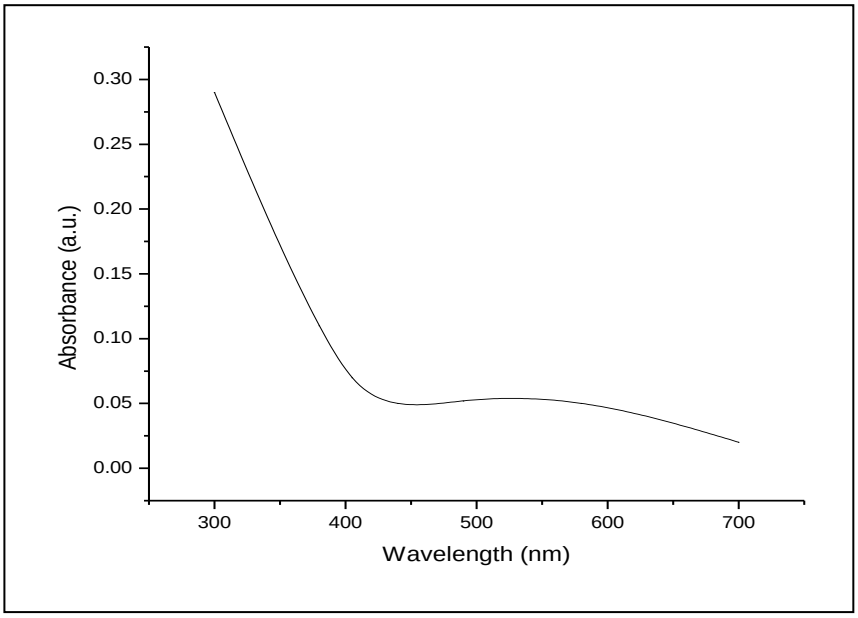

Figure 5.3: UV-Visible spectra of Blank

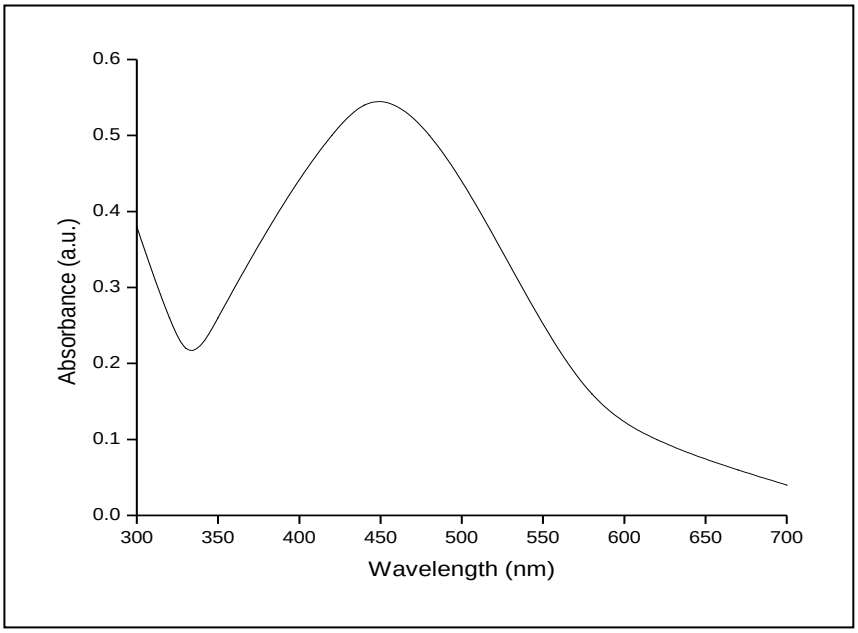

Figure 5.4: UV-Visible spectra of Silver Nanoparticles

5.4.2 Particle Size Analysis of Silver Nanoparticles

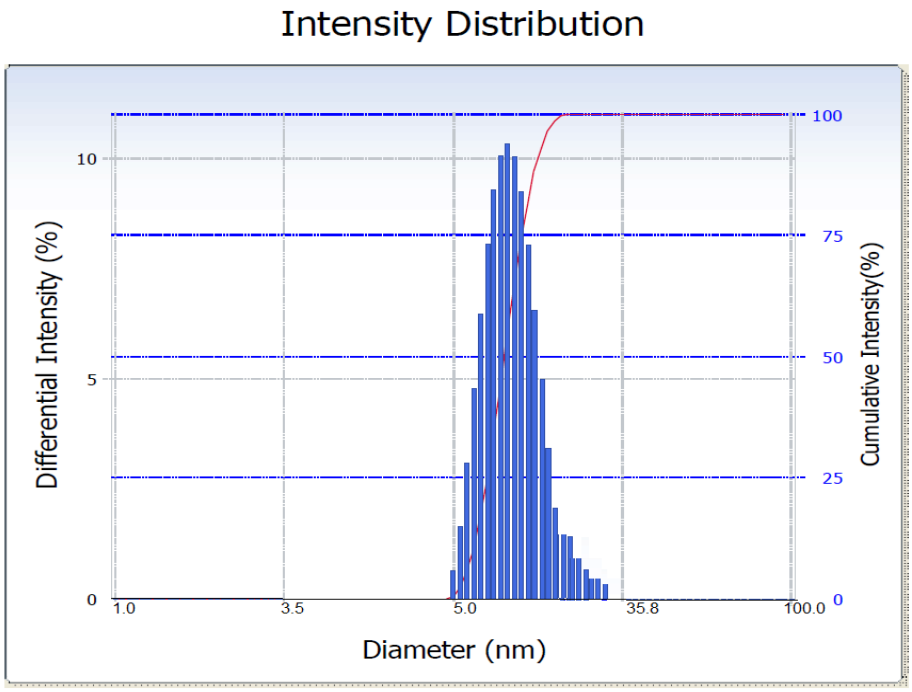

Figure 5.5: Particle Size Analysis of Silver Nanoparticles

Particle size analysis study showed the range of the particles-

- Size range of Silver Nanoparticle 5-30 nm

5.4.3 SEM-EDX Analysis of Silver Nanoparticles

The structural features of the produced gold nanoparticles were characterized using SEM. As the metal particles are good conductors, they can be observed without any prior carbon coating at a magnification of 1000× in a voltage of 10 kV. Fig. 5.7 is the SEM image of *Trichoderma reesei* NCIM biomass after the addition of the chloroauric acid at 1-100 nm. It was identified from SEM images that the mold mycelia loaded with glittering particle. This depicts that the glittering particles on the mycelia should be Silver Nanoparticles accumulated on the mycelia intracellularly.

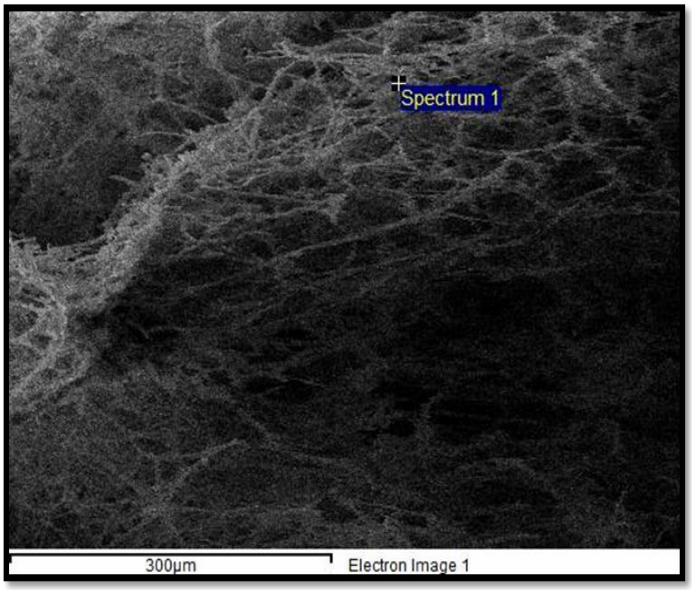

Figure 5.6: SEM image of Silver Nanoparticle

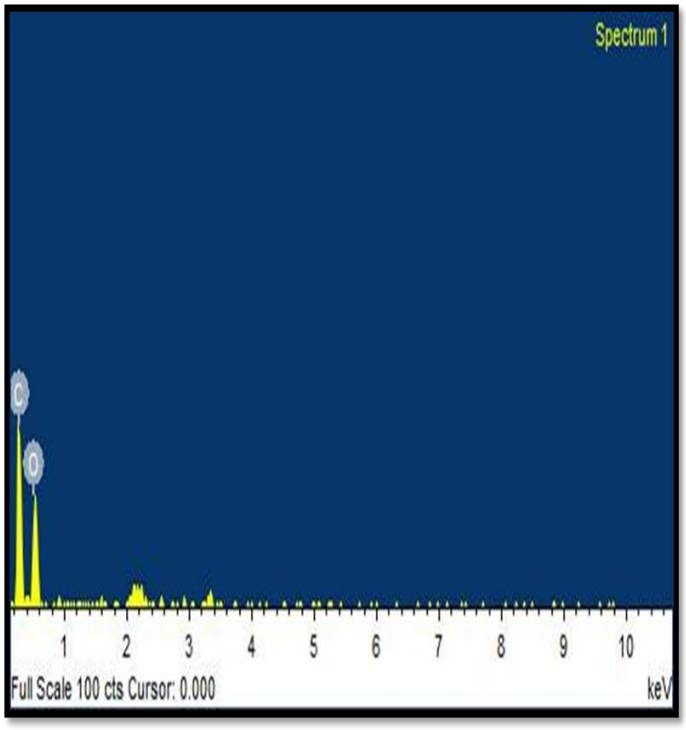

Figure 5.7: EDX Micrograph of Mold

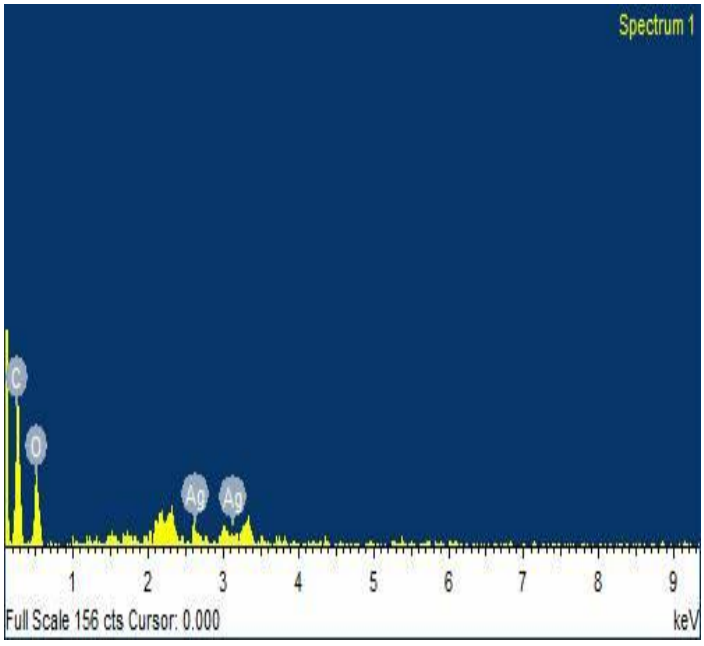

Figure 5.8: EDX Micrograph of Silver Nanoparticle

SEM images concludes that the nanoparticles were identified from shiny particle on the surface of mold cells. The shiny particle observed to be with no definite morphology.

Carbon and Oxygen peaks attribute to the bio-molecules that are bound to the nanoparticles which are acting as stabilising agents. Peaks of Silver in the EDX spectrum appeared around 1.5-3 keV indicating the existence of silver atoms.

5.4.4 TEM Analysis of Silver Nanoparticles

TEM images of Silver nanoparticles clarify that the nanoparticles formed are characterized by uniform distribution with round irregular shape.

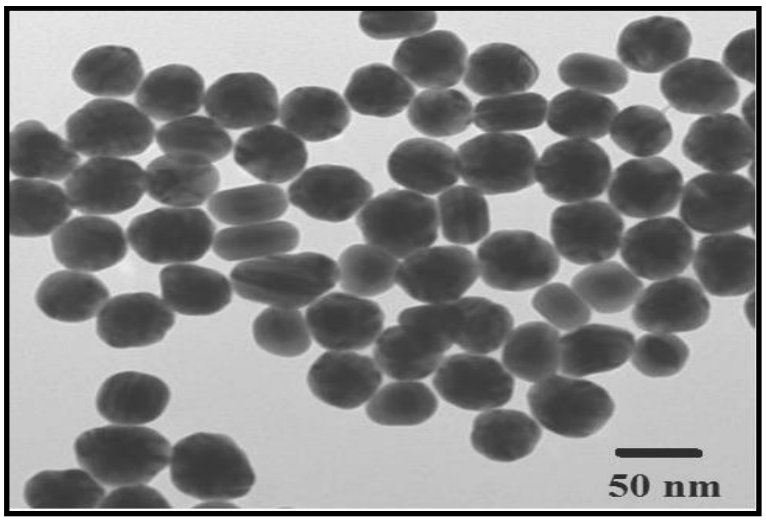

Figure 5.9: TEM image of Silver Nanoparticles