## 5.1 Introduction

Nanotechnology has broad successful applications in the field of molecular biology, medical science as well as target based drug delivery. Nanochemical pesticide formulations with different nanotechnology techniques have been proved successful approach for pest control (Rabinow, 2004). Pesticides in form of nanoencapsulation can enhanced pesticide adsorption to plant (Scrinis, & Lyons., 2007). Also, Barik et al., (2008) reported that use of nano silica with 3-5 nm particles as nanoinsecticide against agriculture insect pest. Also, PEG coated nanoparticles loaded garlic oil was act as biocontrol tool against stored product damaging *Tribolium castaneum* insect (Yang et al.,2009). Moreover, silver nanoparticles have been used in the management of *Aphis nerii* and *Callosobruchus maculates* (Rouhani et al., 2012). Nanoparticles in nanostructures form have potent insecticidal activity against *Sitophilus oryzae* (R) and *Rhyzopertha dominica* (F) (Stadler et al., 2010). Porous silica nanoparticles loaded with validamycin proved to be potent insecticide with control release strategy (Stadler et al., 2010). Bt based synthesized silver nanoparticles in dark condition observed 0.10 ppm ($LC_{50}$) concentration to be larvicidal against *Aedes aegypti* (Diptera: Culicidae) (Banu et al., 2014).

*Spodoptera lituralis* (Lepidoptera: Noctuidae) is one of the major polyphagous pests invading more than 170 plant species. Chemical pesticides have been extensively used for controlling this pest in different crops such as cotton, castor, groundnut, tobacco, mustard, tomato, chilly (Arumugam.,2014). However, the pest developed resistance against almost all commercial available current chemical pesticides (Bharani et al., 2017). Nanotechnology based inorganic nanoparticles such as silver, gold, ZnO and $TiO_2$

nanoparticles have been extensively employed in the treatment of diseases, water treatment and sun screen lotions However, it needs harsh, toxic and expensive chemicals in the production of them (Shankar et al.,2003). Green chemistry has been utilized for the synthesis of silver nanoparticles for many years and proved to be successful in nanotechnological applications. Plant extracts, bacteria, fungi and yeast exploited successfully for extracellular biosynthesis of silver and gold nanoparticles (Sadowski et al.,2010).The main aim of present study is rapid biogenic way synthesis of silver nanoparticles with supernatant of *Bacillus thuringiensis krustaki* HD-73 by sun light irradiation and determination of its insecticidal activity against devastating lepidopteran pest *Spodoptera litoralis* (Lepidoptera: Noctuidae).

**5.2 Material & Methods**

**5.2.1 Sunlight based synthesis of AgNPs with supernatant of *Btk-HD73***

Strain *Btk-HD73* obtained from BGSC, Ohio, USA and grown in 100 ml GYS sporulation medium at 30 °C till sporulation (48-72 h). After sporulation stage, *Bt* culture was centrifuged at 8000 RPM for 5 min and supernatant was employed for the preparation of reaction mixtures. The reaction system was prepared by taking 2.5 ml Bt supernatant, 200 µL of 100mM $AgNO_3$ and make final volume to 20 ml with de-ionised double distilled water. Immediately, the system was exposed to direct sunlight irradiation from time period 10.00 a.m. to 5.00 p.m. during open sky condition. Periodically, aliquots of the reaction mixture were taken periodically an hour interval for UV-Visible spectroscopy.

### 5.2.2 UV -Visible spectroscopy analysis of synthesized AgNPs

The prepared nanoparticles were analyzed by UV-Visible spectroscopy. The aliquot was further diluted with MQ water and the absorption was monitored with UV-visible spectrophotometer Beckman coulter (DU-720) in the range of 290 – 800 nm. Also, negative control-I and II which comprise of supernatant of Bt and $AgNO_3$ respectively in defined under similar condition.

### 5.2.3 FTIR analysis of samples

The fourrier transformed infrared (FTIR) spectra were recorded using Perkin Elmer-RS1 spectrometer in the diffuse reflection mode at resolution of 4 $cm^{-1}$ and all measurements were carried out in the range of 400–4,000 $cm^{-1}$.

### 5.2.4 Transmission electron microscopy (TEM) analysis

Silver nanoparticles were analysed Transmission electron microscopy (TEM) by placing a drop of prepared AgNPs on carbon coated copper grid followed by solvent evaporation. The qualitative characterization of nanoparticles was performed by Philips –Tecnai (Philip electron optics, Holland) electron microscope operated at 200kv.

### 5.2.5 XRD measurement

Silver nanoparticles were purified by repeated centrifugation at 15,000 RPM for 20 min followed by lyophilized. Lyophilized AgNPs used for the confirmation of shape by XRD using Pan analytical, PW, The Netherland operated at 45 KV voltage current 40 mA with Cu anode. The crystalline domain was calculated by using Scherr's formula $d=0.94\lambda/\beta_{0.5}\cos\theta$.

### 5.2.6 AgNPs synthesis in dark condition

In order to compare reaction rate, the reaction mixture prepared with different concentration 10%. 20% and 30 % of Bt supernatant and $AgNO_3$ solution. The reaction mixtures were kept in dark condition at room temperature. Aliquots were taken at 24 h interval and O.D. at 440 nm. recorded.

### 5.2.7 Bioassay of synthesized AgNPS against *Spodoptera litoralis*

AgNPs was evaluated to insecticidal activity against *Spodoptera litoralis* by lead disc method. Silver nanoparticles solution prepared in 0.6 – 4.8 mg/ml. 250 µL volume of each concentration was dried on 5 $cm^2$ castor leave. The leaves were cut to 1 $cm^2$ pieces and allow to fed second instar larvae of *S. littoralis.* Fifteen larvae in triplicate were used for each dose. The bioassay was carried out under 12h light: day condition and 60-70 % relative humidity and mortalities were counted on third, six and ninth day. Probit analysis was performed by SPSS software version 17. 00 (Chicago, IL) was to the determination of $LC_{50}$ value.

## 5.3 Results

### 5.3.1 UV-visible spectroscopy analysis of synthesized AgNPs

The supernatant of *Bt* culture act as a strong reducing agent and form to $Ag^+$ ion from $AgNO_3$. Thus, the formation of nanoparticles was observed by the color change of the solution from pale white to brown with the course of the reaction and ultimately to black (Figure 5.2 a,b). The results indicated that continued reduction from ionic silver to elemental silver occurred in the formation of silver nanoparticles. UV-Visible spectra of AgNps exhibited $\lambda_{max}$ around 440 nm (Figure 5.1) and broadening of peak indicate polydispersion of nanoparticles in solution.

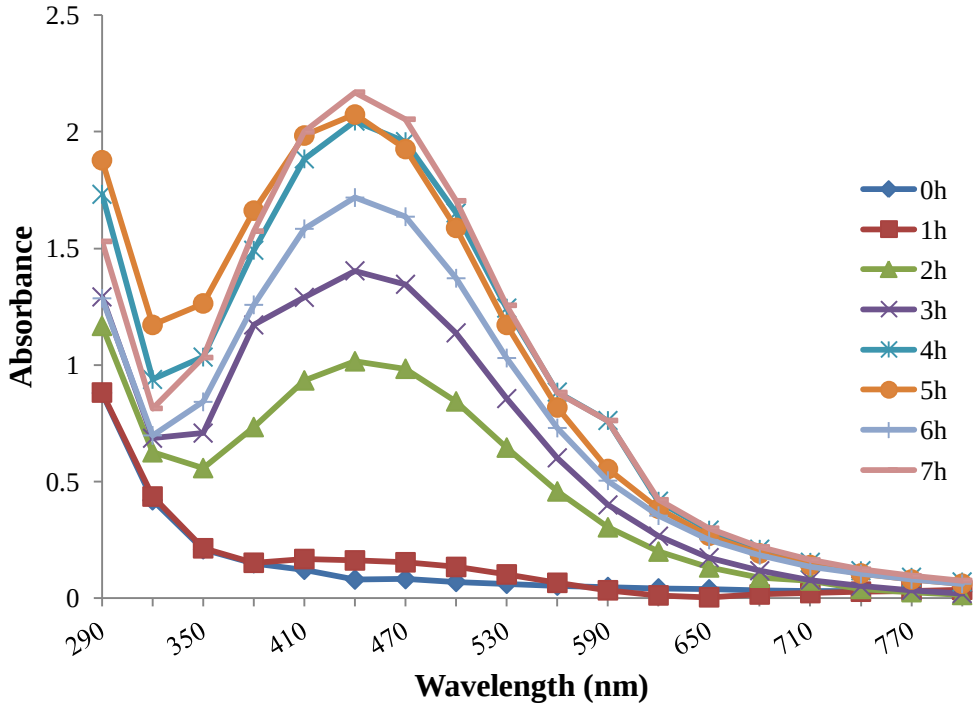


Figure 5.1: UV-Visible spectra of silver nanoparticles synthesized at different time

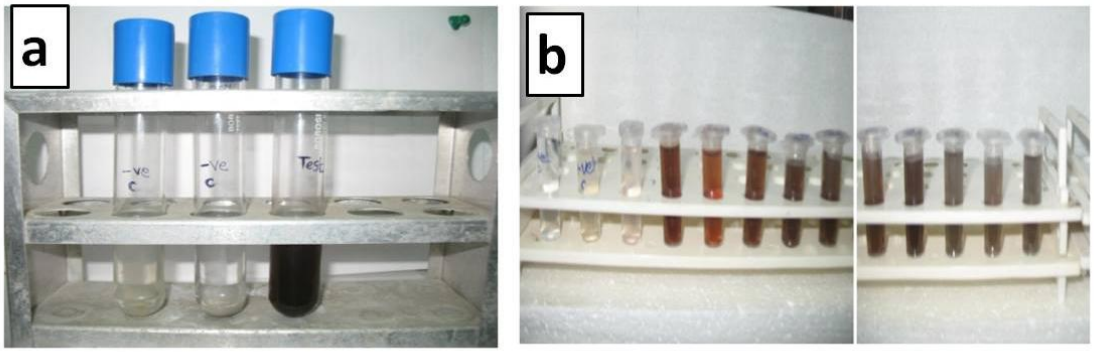


Figure 5.2: Synthesis of silver nanoparticles by *Btk HD-73* (a) sunlight exposed reaction system having supernatant of *Btk HD-73* and $AgNO_3$ solution (blackish) after 7 h compare to colorless controls (b) Different aliquots collected at hour interval and observed color change to reduction of Ag ion.

### 5.3.2 TEM analysis of synthesized AgNPs

TEM image revealed that nanoparticles were of regular surface and spherical, oval in shape with diameter ranging from 50-80 nm (Figure 5.3 a). Capping layer observed surrounding to the nanoparticles (Figure 5.3 b).

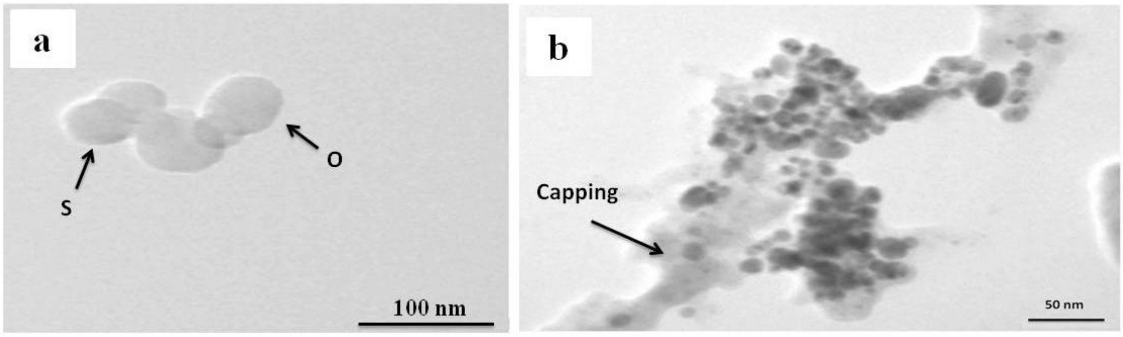


Figure 5.3: TEM image of AgNPs : (a) oval and spherical shape of nanoparticles (b) capping around nanoparticles

### 5.3.3 FTRI analysis of silver nanoparticles

FTIR spectrum analysis revealed that the peak seen at 2917 $cm^{-1}$ was of secondary amines, 1462 $cm^{-1}$ and 1515 $cm^{-1}$ can assigned to be C-N stretching vibration of aromatic and aliphatic amines of aromatic amino acids while bands present at 1599 $cm^{-1}$ and 1527 $cm^{-1}$ correspond to stretch of molecular vibration of N-H bond of proteins molecules (Figure 5.4).

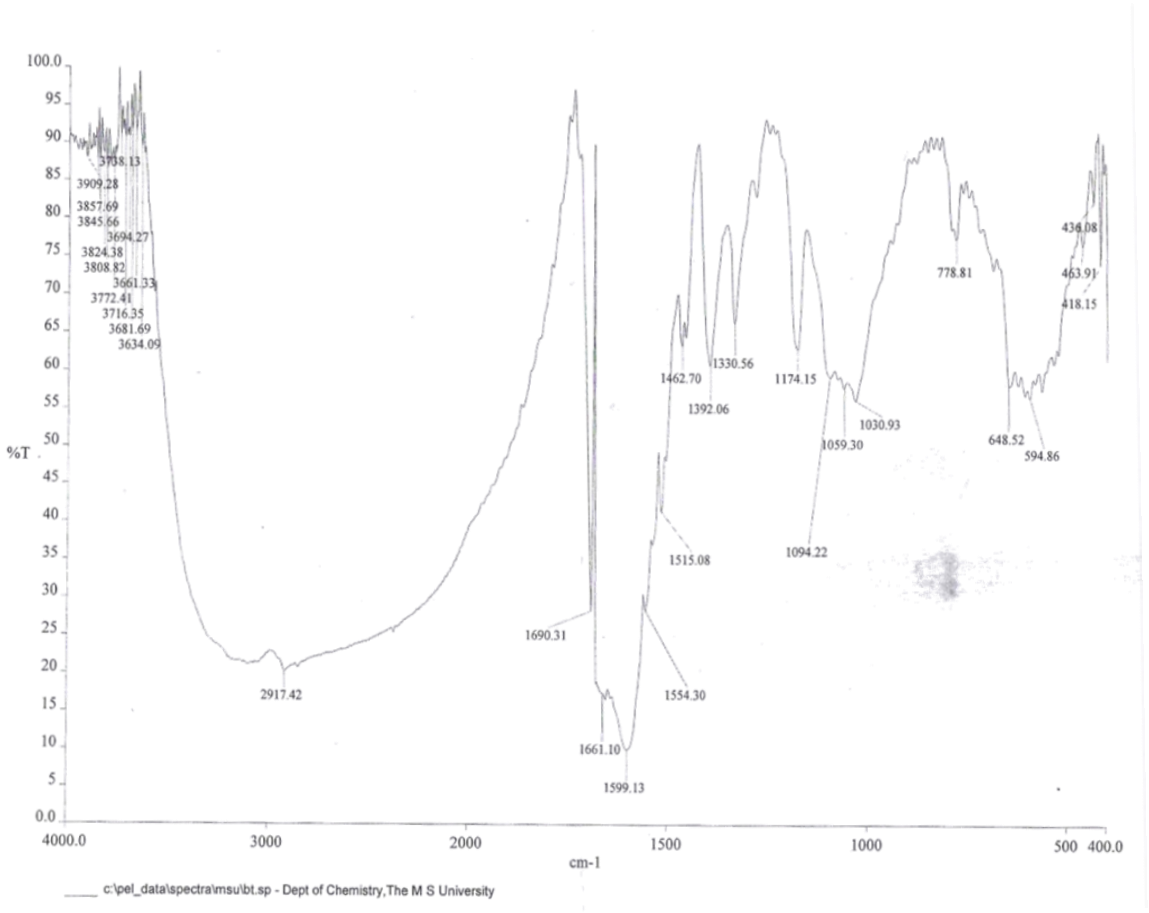


Figure 5.4: FTIR spectrum of synthesized silver nanoparticles

### 5.3.4 XRD analysis of lyophilized AgNPs

XRD analysis showed four distinct diffraction peaks at 38.06°, 44.32°,64.14° and 77.33° and can be indexed 2 θ values of (111), (200), (220), (311) crystalline planes of Ag (Figure 5.5).

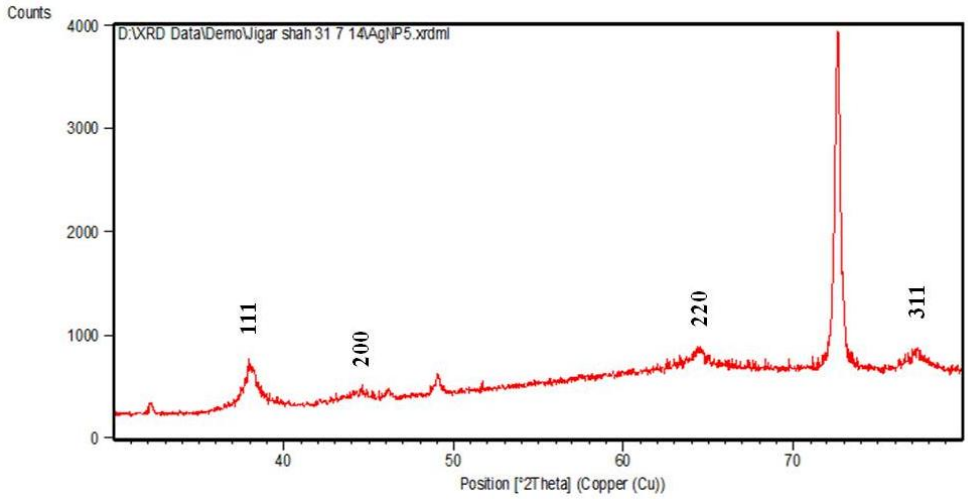


Figure 5.5: XRD patterns of Ag nanoparticles

### 5.3.5 Comparative analysis of formation of silver nanoparticles in dark condition

It has observed that formation of AgNPs occurred in dark condition with different concentration of reducing agent of Bt supernatant. However, the O.D. reached only 0.3 to 0.4 after 24 h incubation time and remained steady even further incubation (Figure 5.6).

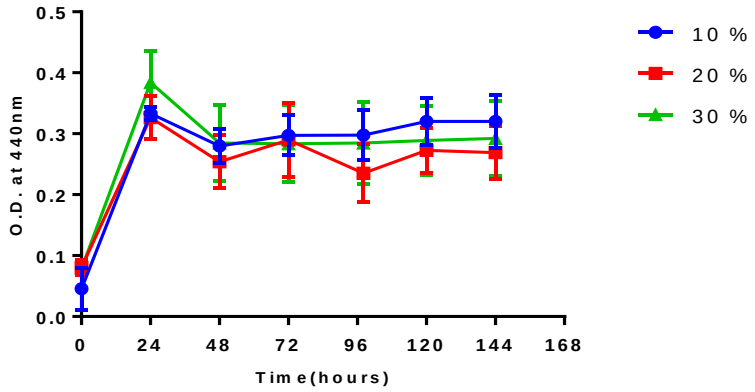


Figure 5.6 silver nanoparticles synthesis in dark condition

### 5.3.6 Insect bioassay of silver nanoparticles against *S.litoralis*

Table 5.1: Insecticidal activity of silver nanoparticles against *Spodoptera litoralis*

| AgNps (mg/ml) | Average No* of *S.litoralis* larvae survive / dose and %# mortality after indicated days | | | | | |
|---|---|---|---|---|---|---|
| | Third day | | Sixth day | | Ninth day | |
| | No* | %# | No* | %# | No* | %# |
| 0.60 | 10.66 ± 1.52 | 27.29 | 10.33 ± 1.15 | 26.43 | 8.00 ± 1.00 | 41.43 |
| 1.20 | 10.00 ± 1.00 | 31.79 | 9.00 ±1.00 | 35.71 | 6.66 ± 0.57 | 51.24 |
| 2.40 | 8.66 ± 0.57 | 40.93 | 7.66 ± 1.52 | 45.29 | 5.66 ± 0.57 | 58.57 |
| 4.80 | 7.66 ± 0.57 | 47.75 | 6.00 ± 1.00 | 57.14 | 2.33 ± 0.57 | 82.94 |
| - ve control | 14.66 ± 0.57 | ____ | 14.00 ± 0.00 | ____ | 13.66 ± 0.57 | _____ |

No*: Average No of larvae survive
# : corrected mortality

The results of insect bioassay with silver nanoparticles showed the insecticidal activity property to *Spodoptera litoralis* (Table 5.1). 2.4 and 4.8 mg/ml AgNPs concentration would act as strong larval population control and $LC_{50}$ was found to be 1.348 mg/ml (95% confidence interval 0.784 -1.560 mg/ml) on ninth day of the experiment.

## 5.4 Discussion

Although the development of chemical and physical techniques for silver nanoparticles synthesis have been reported, most methods deal with extensive consumption of energy to maintain high temperature and pressure in the synthesis process. Synthesis of nanoparticles by green chemistry has been proved to be an approach successful way for the production of nanoparticles. Biological methods is an excellent alternative to physical

and chemical methods because of their low cost downstream processing and eco-friendly preparation. The $Ag^{+}$ ion reduced to $Ag^{0}$ by gain of electron might be from reductase enzyme (Kalimuthu et al., 2008). Sastri et al., (1998) demonstrated the color transformation from light yellow to brown is indication of AgNPs synthesis because the color change of solution of AgNPs was due to the excitation of surface plasmon vibration in metal nanoparticles. Also, *Bacillus* strain (SC-11) based extracellular synthesis of AgNPs reported after 24 h incubation with $AgNO_3$ (Das et al., 2014). Banu et al., (2014) reported that to Bt supernatant based synthesis of silver nanoparticles occurred after 72 h of incubation in dark condition. During present study, the maximum intensity of peak $\lambda_{max}$ recorded to > 2.0 a.u. in 5 h reaction which indicates very fast formation of AgNPs. Rajasekharreddy et al., (2010) reported that different plants extracts could synthesiz silver nanoparticles at different time such as *C.gigantea* plant extracts synthesis in 154 min, *T.procumbens, J.cuecus* extract in 25 min which supports current present method for synthesis of silver nanoparticles.Also, it is very simple process to produce silver nanoparticles and can be extended to large scale production.

TEM results revealed that nanoparticles with adequate size in nano scale and presence of capping layer surrounding nanoparticles. FTIR analysis interpreted that capping material to be protein in nature. Biological molecules performed dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Mallikarjuna et al., 2011). Raut et al., (2014) reported proteins present in supernatant functioning as capping agents as the carbonyl group from the amino acid residues show stronger ability to bind to metals.

XRD analysis indicates the structural characteristic pattern confirms the AgNPs have crystalline structure and the additional peak at around 72 ° might be attributable to the proteins which are capped with the AgNPs (Shankar et al., 2004; Raute at al.,2014) Insecticidal effect of AgNPs against devastating pest *Spodoptera litoralis* concluded that larval population could control effectively. This will open new approach of application of silver nanoparticles with Bt toxin to combat insect resistant management.

**5.5 References**

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