BIOSYNTHESIS OF SILVER NANOPARTICLES USING THE CULTURE SUPERNATANT OF CYANOBACTERIA *Pharmidium fragile* AND ITS BIOCIDAL EFFECT ON *Xanthomonas oryzae.*

1.Introduction

The word nano is used to indicate one billionth of a meter or $10^{9.}$ Nanotechnology has emerged as integration between biotechnology and nanotechnology for developing of biosynthetic and eco -friendly technology for synthesis of nanomaterials. Nanoparticles are clusters of atoms in the size range of 1- 100 nm. The metallic nanoparticles are most promising as they show good antibacterial properties due to their large surface area to volume ratio, which is coming up as the current interest in the researchers due to the growing microbial resistant against metal ions, antibiotics, and the development of resistant strains.

The physical and chemical methods for the synthesis of silver nanoparticles include chemical reduction of silver ions in aqueous solutions with or without stabilizing agents, thermal decomposition in organic solvent, chemical and photo-reduction in inverse micelles and radiation chemical reaction. Due to rapid progress of application of nanomaterial in different fields, there is a growing need to develop clean, non-toxic ,and environmental friendly procedures for the synthesis and assembly of nanoparticles (NPs). Previous literature revealed that the nano particles synthesis using micro algae as source has been unexplored and underexploited. Recently there are a few reports that microalgae is being used as a biofactory for synthesis of metabolic Nanoparticles

In the present study the culture supernatant of the cyanobacteria *Pharmidium fragile* was used to synthesis of silver nanoparticles and it was characterized by UV-visible spectroscopy,XRD,and FTIR. The synthesised nanoparticle was used for the study of bactericidal assay against rice blight bacterial pathogen *Xanthomonas oryzae.*

2.Objectives

1. To synthesis Silver nanoparticles using the culture supernatant of Cyanobacteria *Pharmidium fragile* as reducing agent.

2. To characterize the synthesised nanoparticles by UV-visible spectroscopy, XRD,And FT IR.

3. To study the bactericidal effect of nanoparticles against the blight disease pathogen of rice *Xanthomonas oryzae.*

3.Methodology

The culture supernatant of *Pharmidium fragile was* filtered through Whatman number 1 filter paper, and the filtrate was then centrifuced at 5000 rpm for 5 minutes and transfer to in 250 mL, Erlenmeyer flash and stored at 4^0c for further experiments. The filtrate is used as a reducing and stabilizing agent for 1mM silver nitrate ($AgNo_3$) solution. The reduction of pure Ag+ ions was monitored by obtaining the UV-Visible spectrum of the reaction mixture at various time intervals via 1hr, 2hr, 3hr, 4hr and 5hr. by scanning the absorbance spectra in 250-650 nm range of wavelength. The freeze- dried, powdered silver nanoparticles were used for FTIR and XRD analysis (Jain *et al.*, 2009).

The bacteria Xanthomonas oryzae was used for the experiment. The pathogen was sub-cultured on nutrient agar and disc diffusion assay was performed to record the dose dependent assay of nanosilver on test bacteria. Bacterial inoculum was prepared by growing a single colony overnight in nutrient broth and the log phase culture of *Xanthomonas oryzae* was swabbed over the agar plates. Then the Nanoparticles coated disc, and some antibiotics disc were plated over the nutrient agar plates then the plates were incubated at 24hrs 30˚c .After incubation clear zone around the disc was an evidence of antibacterial activity. Diameter of the zone of inhibition was measured as mm. Each test will be performed in triplicate.

RESULT AND DISCUSSION

4.1 Formations of silver nanoparticles

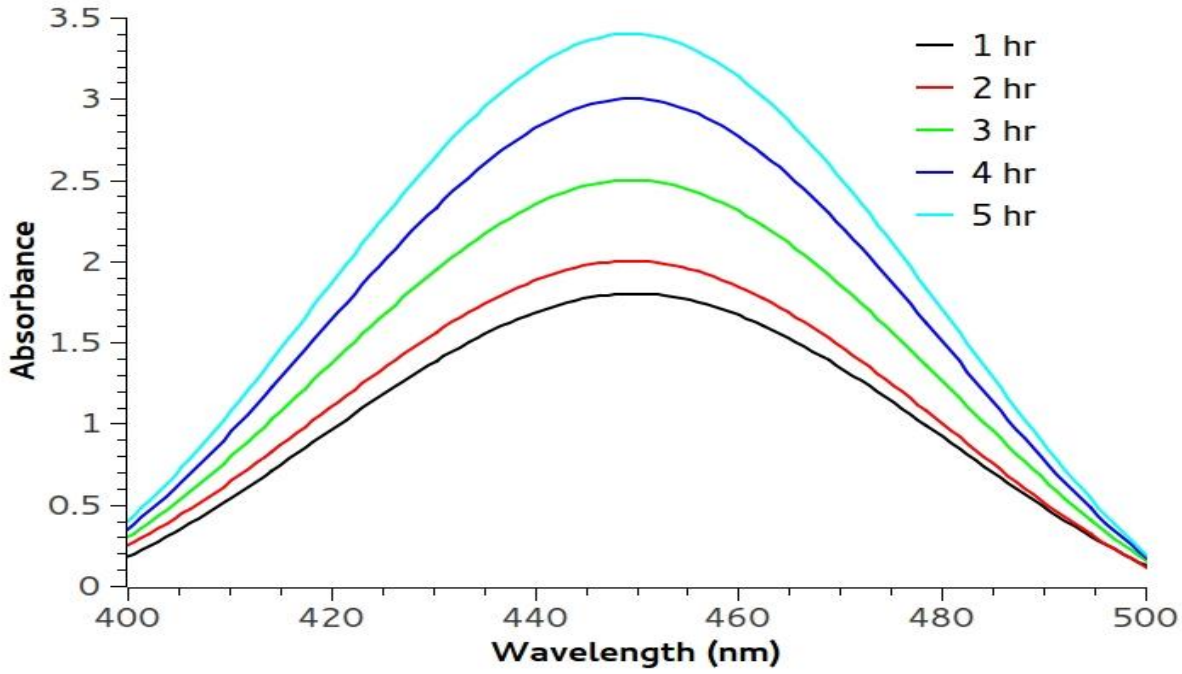
The formation of silvernanoparticles by the culture supernatant of *Pharmidium fragile* was investigated.Fig.2 shows two conical flasks with the culture supernatant of *Pharmidium fragile* after reaction with the Ag+ ions for 12 hours.it was observed that the supernatant has no change in colour. Before reaction with the silver ions (Left flask) ,which changes to a browinish colour on completion of the reaction (right flask) .The appearance of a yellowish –brown colour in solution containing the biomass is a clear indication of the formation of silver nanoparticles in the reaction mixture and is due to the excitation of surface plasmon vibrations in the nano particles (Sastry *et al* .,1998).

Fig .2. Conical flasks with the aqueous extract before (left flask) and after reaction with Ag+for 12 hrs(right flask).

4.2 UV-Visible spectroscopy

The silver nanoparticles are characterized by UV-Visible spectroscopy. The UV-visible spectra recorded the culture supernatant *of pharmidium fragile* in reaction medium at different time intervals of reaction is plotted in a graph (Fig.3).It was observed from the spectra that the silver nanoparticles surface plasmon resonance

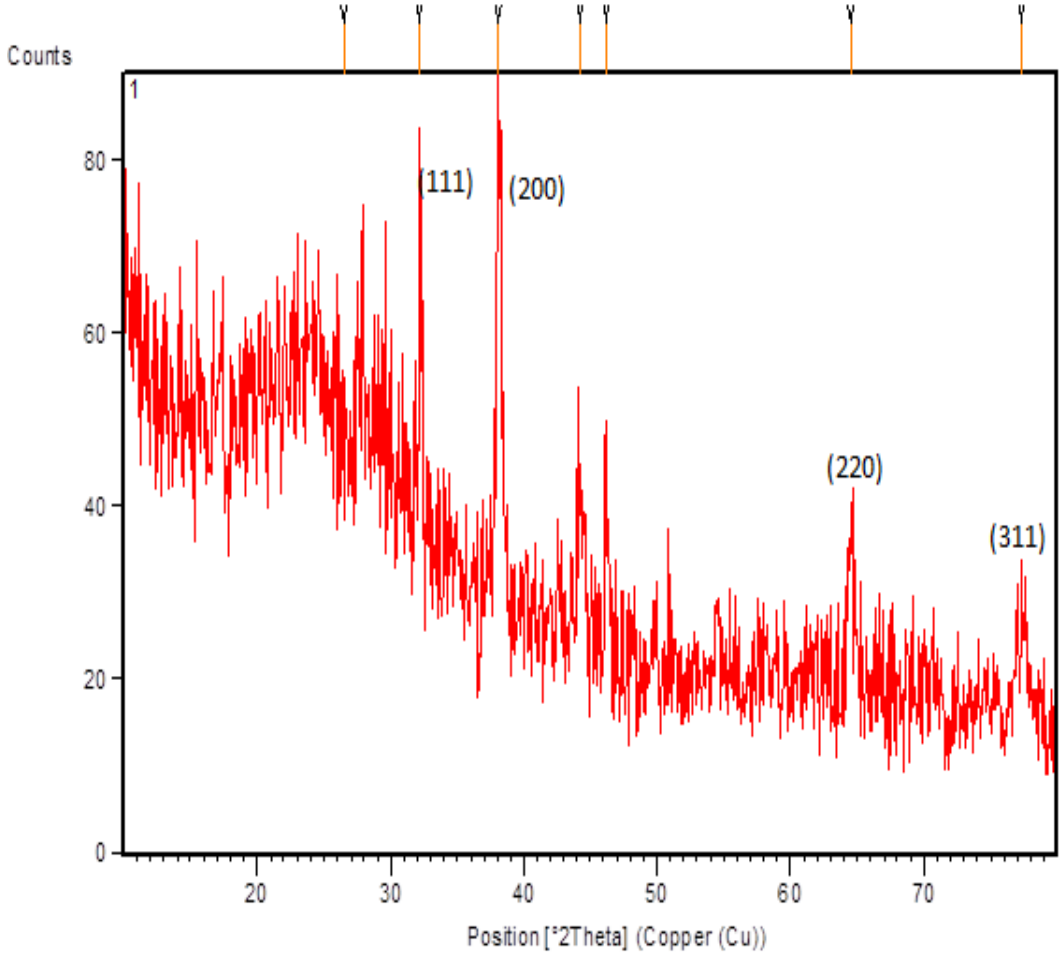
peak occurred between 410-420 nm.UV-Visible spectrograph of the colloid of Ag nanoparticles has been recorded as a function of time by using a quartz cuvette with silver nitrate as the reference. The technique has proved to be very use for the analysis of metal nanoparticles`(Sastry *etal.*,1998)



4.3 X-ray diffraction Analysis

Synthesis of silver nanoparticles by the culture supernatant of *pharmidium fragile* was further confirmed by the characteristic XRD Peaks (Fig.4).The XRD pattern shows seven peaks with intense peaks in the whole spectrum of 2θ values ranging from 32.19 to 77.29(Table. 1).XRD spectra of pure crystalline silver structure have been published by the joint committee on powder diffraction standards (file no.04-0783).The diffraction at the 32.190◦, 38.07◦ ,64.50˚,and77.29˚ can be indexed to the (111), (200), (220) and (311), planes of the face- centered cubic (fcc) silver, respectively .

The full width half maximum (FWHM) values measured for 111,200, 220,and 311 planes of reflection were used with Debye-Scherer equation to calculate the size of the nanoparticles. The average size of the particles synthesized was 2.6 nm with size range from 0.023 nm to 2.2 nm. Wide angle X-ray diffraction (WAXs) was carried out for determine the structure and the par ticle size of nanoparticles. The broadening of Bragg peaks representative of face centered cubic (fcc). Sathyavathi *et al* ., 2010).The XRD pattern thus clearly showed that the nanoparticles formed by the culture



supernatant of *Pharmidium fragile* are crystalline in nature (Huang *et al*.,2007).

fig.4.XRD Pattern of synthesized silver nanoparticles(peaks corresponding to silver).

Table 1. Size of the NPs synthesized by the extract of *Pharmidium fragile*.

Pos(2T◦h)	Height (cts)	FWHM Left(2Th◦)	d spacing (A◦)	Rel.int(%)	Size of Silver nanoparticles(nm)
6.5(2)	14(2)	8(1)	3.35956	27.63	0.023
32.190(8)	49(6)	0.07(2)	2.77854	100.00	2.2
38.072(2)	42(5)	0.39(7)	2.36212	85.59	0.38
44.25(4)	14(2)	0.6(1)	2.04505	28.35	0.25
46.21(3)	14(3)	0.32(8)	1.96313	28.27	1.00
64.50(5)	10(2)	0.6(1)	1.44349	21.21	0.36
77.29(7)	9(2)	1.0(2)	1.23343	17.57	0.25
Average size of the Nano particle is = 0.63					

4.4 FTIR Analysis

FTIR analysis was carried out to identify the possible interaction between silver ions with

aqueous extract of *Pharmidium fragile* is shown in Fig.5.The small peak at 3919.08, 3779.94, 3692.97 were corresponding to O-H Sterching .The peak at 3409.02

represent N-H (amines,amides) . The small peaks at 2921.83 represent C-H (in alkanes), C=N respectively.The strong brond peaks at 1597.68 and 1383.15 were

corrsponding to C=C, C-H (in alkanes) respectively. The broad bond at 1026.25 represent C-O (either, alchol etc.). The position of these bonds are close to that repored for native proteins (Gole *et a*l.,2000).

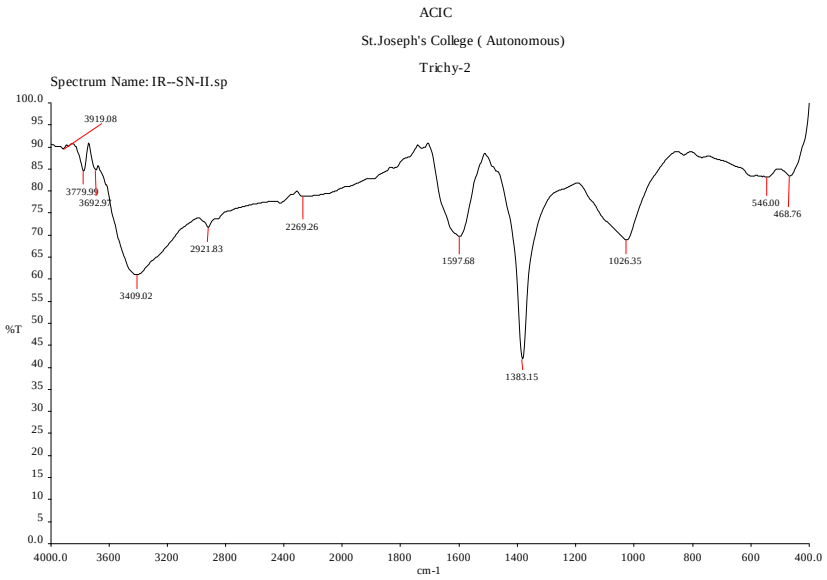


Fig.5. FTIR of the band representing various functional groups.

4.5 Bactericidal assay

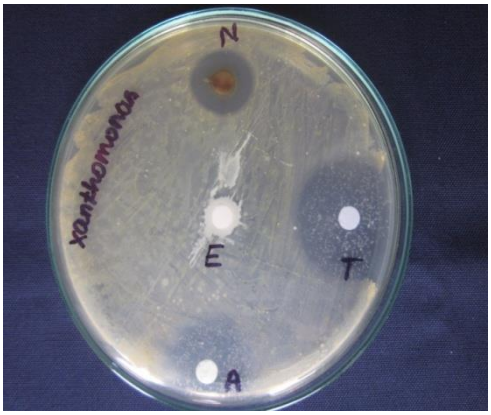
The antibacterial activity of synthesised nanoparticles and commercially available antibiotics were carried out against *Xanthomonas oryzae.*The silver nanoparticle showed considerable clear zone of inhibition against *Xanthomonas oryzae.* The zone of inhibition of commercial antibiotics like Tetracyclin and Ampicillin disc showed inhibition hallow of 3.8mm and 2.9mm respectively (Table 2and fig .6).

The zone of inhibition was described in mm and mean value of Triplicate. The efficiency of various silver based antimicrobial agent in polyamide toward their silver ions release in an *aque*ous medium was also investigated and discussed . The efficiency of various silver based antimicrobial agent in polyamide toward their silver ions released in aquous medium was also investigated and discussed in number of plants including algae, yeast, fungi(Arya,2010).

Table:2 Zone of inhibition (mm) of Nano particle against Xanthomonas oryzae.

S.NO	Name of the organism	Nano particles 10µg/ml	Antibiotics	
			Tetracyclin 10µg/ml	Ampicillin 10µg/ml
1.	*Xanthomonas oryzae*	2.7	3.8	2.9

Fig: 6 ***Xanthomonas oryzae***



N- Nanoparticles T – Tetracyclin A – Ampicillin E- Plant Extract

5.Conclusion

In the present study we investigated a simple biological process for synthesizing silver nanoparticles using the culture supernatant of *Pharmidium fragile.* The characterization of Ag+ ions exposed to the leaf extract by UV- visible spectroscopy, XRD,techniques confirmed the reduction of silver ions to silver nanoparticles. The FTIR analysis suggested that the capping over Ag-Nps .The synthesized nanoparticles showed effective antibacterial activity against *Xathomonas orizae.* Hence this method is cost effective and utilized for many biological applications.

Summary:

The environmental friendly process for the synthesis of nano particles is evolving into an important branch of nano technology. Traditionally metallic silver nanoparticles are synthesised by wet chemical synthesis techniques. Such approach uses toxic chemicals. The present study deals with the alternative,cost effective and green approach. The culture supernatant of *Pharmidium fragile* is used for the reduction silver ions. Synthesis of silver nano particles are usually observed by change in coiour of the reduction medium . The colour of the medium change into brown colour .

In UV visible spectro scopy ,the peak value increases between 440-460 nm rang , indicating the biosynthesis of nano particles. Further , the synthesised silver nano particles are confirmed by XRD.The peaks at 32.19, 38.07, 64.50 and 77.29 indicates silver nano particles .

The average size of the nanoparticle are calculated using Debye scherer formula . It was calculated that the average size of nano particles are 0.63 nm . The antibacterial activity of the synthesised nanoparticles are carried out against *Xanthomonas oryzae* . it was found that the synthesised nano particle show inhibitory effect on *Xanthomonas oryzae* , the zone of inhibition is 2.7 mm.