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Biosynthesis of Silver Nanoparticles by *Chlorodesmis Hildebrandtii A. Gepp* & *E. Gepp* Including its Agricultural and Biomedical Implications

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Abstract

The aim of this work was to investigate the biosynthesis of Silver (Ag) Nanoparticles from aqueous extract of green seaweed *Chlorodesmis hildebrandtii* and its characterization. The synthesized Ag-Nanoparticles were characterized using UV-Visible Spectrophotometer, Fourier Transform Infrared Spectroscopy, Dynamic Light Scattering, Scanning Electron Microscopy and X-Ray Diffraction analysis. The biosynthesized Ag-Nanoparticles had been analysed for its inhibitory activity against some human pathogenic bacteria. The biosynthesized Ag-Nanoparticles had been tested for its potentiality to seed germination to confirm its Phyto-friendly nature and for its future use as bio-nano-fertilizer. The antibacterial activity was tested with agar disc diffusion method and the seed germination was also done according to standard protocol. The inhibitory activity of biosynthesized Silver Nanoparticles was the highest against *Klebsiella pneumoniae* (1.5±0.5cm), followed by *Escherichia coli* (0.63±0.05cm) and *Proteus mirabilis* (0.23±0.05cm) than the zone of inhibition of antibiotic Chloramphenicol (5mg/ml). The biosynthesized Ag-Nanoparticles had accelerating potentiality to seeds germination. The seed germination percentage was more in case of seeds treated with biosynthesized Ag-Nanoparticles. The seed germination was 100 percent for both seeds of *Abelmoschus esculentus* and *Raphanus sativus* var. *longinnatus*. But seed germination percentage was less in case of seeds treated with normal water (60%), seaweed extract (60%) and seaweed liquid fertilizer (40%).

Keywords: Seaweed; Biosynthesis; Silver Nanoparticles; Seed Germination; Antibacterial Activity

Introduction

It had been reported that chemicals originated Ag-Nanoparticles, due to its unique physical and chemical properties especially very quick penetration with its nanosized, had adverse effect to the environment and in human health. From various industrial effluents, tonnes of free silver ions had been released in to the aqueous phase

which had serious adverse effect on human health such as permanent bluish-gray discolouration of the skin (argyria) and the free silver also formed some compounds which caused kidney and liver damage, skin, respiratory, eye and intestinal tract irritations and the also the changes in blood cells [1]. Some previous research showed that some seaweed had excellent activity for plant growth promotion as bio-nano-fertilizer. Such as the bio-nano-fertilizer biosynthesized by the brown seaweed *Sargassum cinctum* promote 80% seed germination for *Abelmoschus esculentus* [2], likewise *Chaetomorpha antennina* biosynthesized Ag-Nanoparticles promote 80% seed germination for *Abelmoschus esculentus* and 75% seed germination for *Raphanus sativus* var. *longipinnatus* [3], *Sargassum illicifolium* biosynthesized Silver Nanoparticles germinates 60% seeds of *Abelmoschus esculentus* and 60% seeds of *Raphanus sativus* var. *longipinnatus* [4] and biosynthesized Silver Nanoparticles by *Amphiroa anceps* germinated 80% seeds of *Abelmoschus esculentus* and 80-85.71% seeds of *Raphanus sativus* var. *longipinnatus* [5]. In this study, we selected green algae *Chlorodesmis hildebrandtii* for biosynthesis of Silver Nanoparticles and its application for potential for seed germination, trying to developed the most potent Nano-bio-fertilizer for agricultural application to promote the growth and yield.

Materials and Methods

Synthesis of Silver Nanoparticles

Seaweed extracts preparation: The fresh seaweed had been collected from Olaikuda (09°19.700N & 079°19.072E), Rameshwaram, south-east coast of India. Seaweed was identified with standard taxonomic key of CMFRI. It was washed with *in-situ* sea water and distilled water thrice.

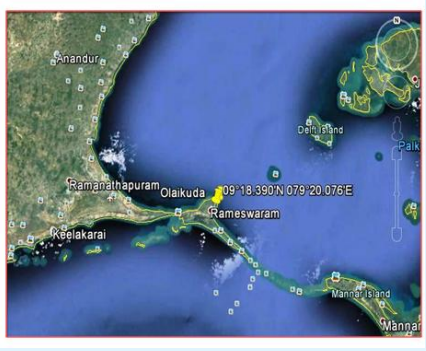


Figure 1: Map showing the sampling location.

Figure 2: Showing *Chlorodesmis hildebrandtii,* Division-Chlorophyta, Order-Bryopsidales, Class-Chlorophyceae and Family-Udoteaceae.

Then, 20 gm of seaweed was cut into very small pieces and grinded to make it powder and was dissolved into 100 ml of distilled water and boiled for 10 minutes. The crude extract of seaweed was filtered with Whatman No. 1 filter paper and repeatedly filtered with thin layer of cotton to get clear seaweed extract. This crude seaweed extract was stored in 4° C for further use (Figures 1 & 2).

Synthesis of Ag-Nanoparticles: The aqueous 1mM $AgNO_3$ solution was prepared with Silver nitrate. For typical synthesis of Silver Nanoparticles, 10 ml of the aqueous extract of seaweed was added to the 90 ml aqueous solution of Silver Nitrate in 250 ml conical flask and kept in room temperature for 72 hours within mechanical shaker at 120 rmp. The colour change of solution indicated the formation of Silver Nanoparticles.

Characterization of Ag-Nanoparticles

UV-Vis Spectrophotometer: After 72 hours synthesis of particles, for characterization, the solution was scanned (300-700nm) with UV-Vis Spectrophotometer (UV-2600 SHIMADZU) and distilled water was used as blank.

Fourier Transform Infrared (FT-IR) Spectroscopy: After synthesis of particles, the solution had been centrifuged at 5000 rmp for 30 minutes to precipitate the pellet of particles at the bottom, then the supernatant were removed and pellet collect and dried at room temperature to make dry powder. The chemical composition of the seaweed was characterized by Perkin Elmer FTIR model 2000. The 1 mg of dry powder of particles was mixed with KBr and made it pellet and used for FT-IR analysis at KBr mode.

Scanning Electron Microscopy: The dry powder of sample was analysed using JEOL JSM-5610LV Scanning Electron Microscope. Thin films of the sample was prepared on a gold coated copper grid by just spraying a very small amount of the powder sample on the grid; and then the film on the SEM grid was allowed for observation.

X-Ray Diffraction Measurements: The dried biosynthesized Silver Nanoparticles were sprayed on measurement cuvette and measured under BRUKER D8 ADVANCE POWDER X-ray diffractometer. The samples were analysed from 20 to 80° theta (θ) range and the operating voltage was 20 KV.

Test on seed germination: The seeds of *Abelmoschus esculentus* (Family-Malvaceae) and *Raphanus sativus var. longinnatus* (Family – Brassicaceae) were dipped within 5% Sodium hypochlorite solution for 15 minutes to ensure seed surface sterility and soaked with Silver Nanoparticles solution for overnight and seeds were also soaked for overnight with normal tape water as control. Then, each piece of filter paper was wetted with 5 ml Silver nanoparticles solution and placed in the Petri plates. The treated seeds were kept on filter paper within Petri plates. Then Petri plates were covered and incubated at room temperature. After 12 hours germination halted and the germination percentage, mean germination time, germination index, relative root elongation, relative seed germination and germination rate were estimated. Germination parameters were calculated using mentioned equations in some literatures [6-9].

Antimicrobial activity: Antibacterial activity of synthesized Ag-Nanoparticles using aqueous extract of *Halimeda gracilis* was assayed by agar disc diffusion method against six human pathogenic bacteria such as *Klebshilla pneumonium, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Proteus mirabilis* which were collected from Department of Medical Microbiology, Raja Muthiah Medical College, Annamalai University. The bacterial cultures were freshly prepared in nutrient broth which was sub-cultured from pure culture. After 24 hours of culture, each bacterial culture was inoculated into the agar plates and kept for 24 hours. The market available Chloramphenicol antibiotic was used as positive control. The 500 mg powder Chloramphenicol was dissolved in 100 ml autoclaved distilled water to made concentration of 5 mg/ml. The 1 mM $AgNO_3$ solution was used as negative control. The 20µl of seaweed synthesized silver nanoparticles solution, solution of negative control and positive control was given to sterile paper discs and the discs were placed on bacterial plates. After 24 hours of incubation, the zones of inhibition were measured in triplicates from three different plates.

Statistical Analysis

Mean and standard deviations were derived from measurements on three replicates for each treatment and the related controls for biochemical composition and total phenol.

Results and Discussions

Synthesis of Silver Nanoparticles

It is well known that Ag-NPs exhibit reddish-brown in water [10]. The mixing of seaweed aqueous solution with Silver Nitrate (1mM) produced dark brownish colour in compare to control Silver nitrate solution and the aqueous seaweed solution which suggested the formation of Ag-NPs by reduction of the aqueous Ag+ (Figure 2). Due to the surface Plasmon vibrations among the produced silver nanoparticles, the color change occurred [11] (Figure 3).

Figure 3: Showing the biosynthesis of Ag-Nanoparticles.

Characterization of Synthesized Nanoparticles

The broad stretch of peak at 448.5 nm (OD-0.856) indicated the synthesis of Silver Nanoparticles (Figure 4).

The FT-IR analysis was done for normal seaweed distilled water extract and the biosynthesized Silver Nanoparticles to know the present functional groups. The stretches and bends at 3410.47 cm^{-1} indicated the presence of amine and amino groups, the broad and flat

stretch at 2735.08 cm^{-1} was produced due to presence of acid (O-H) and aldehydes (C=O), including the presence of alkenes group for the peak produce at 993.42 cm^{-1} in IR spectra. But the IR spectra of the biosynthesized Silver Nanoparticles showed several peaks and bends at several wavelengths which indicated the presence of more functional groups than the seaweed aqueous extract.

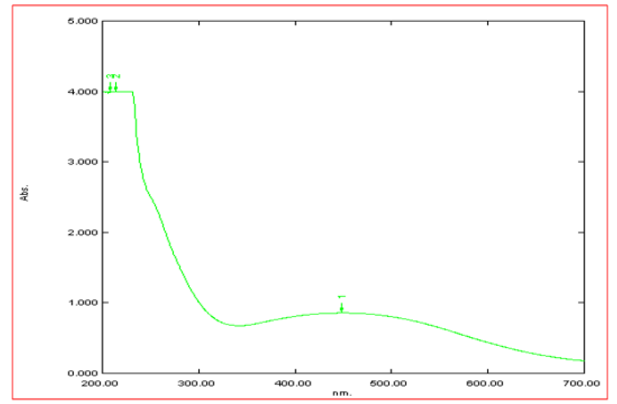


Figure 4: Showing UV-Vis Spectra of synthesized Ag-Nanoparticles.

The broad stretch at 3448.15 cm^{-1} revealed the presence of amine (N-H) or alcohol (O-H) group, bend at 2920.20 cm^{-1}, which for the presence of alkanes (C-H), the stretch at 2849.30 cm^{-1} produced due for the presence of aldehydes group, 1631.74 cm^{-1} peaks for the presence of alkenes (C=C) and amide (N-H), especially the broad peak at 1382.55 cm^{-1} produced for nitro group (N-O), 1019.92 cm^{-1} indicated for the presence of ester group or may be for alkyl halide. The comparative results showed that biosynthesized Silver Nanoparticles had nitro group but the same peak was not present in IR spectra of the aqueous extract of the seaweed (Figures 5 & 6). The scanning electron microscopic images showed the presence of high density, spherical shaped and well distributed Silver Nanoparticles biosynthesized from *Chlorodesmis hildebrandtii* aqueous extract and the size of biosynthesized Silver Nanoparticles was ranged from 10 nm to 100 nm (Figure 7).

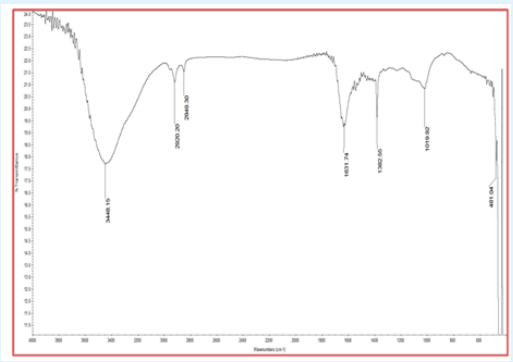


Figure 5: Showing FT-IR Spectra of Silver Nanoparticles.

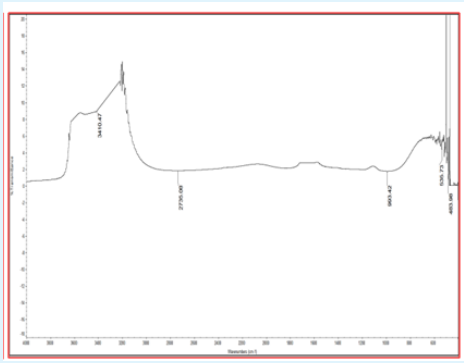


Figure 6: Showing FT-IR Spectra of aqueous extract.

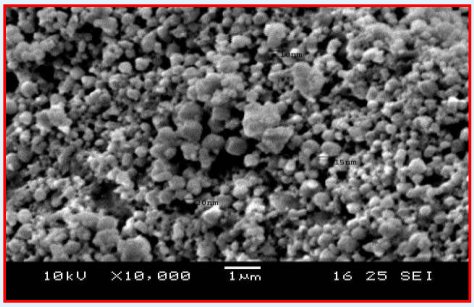


Figure 7: Showing SEM images of biosynthesized Silver Nanoparticles.

Dynamic Light Scattering Study

The Z-average size distribution of Ag-Nanoparticles was found 256.2 d.nm and particles were well distributed in the water solution (Figure 8). The zeta potential value of Ag nanoparticles, - 28.6 mV, indicated the high stability of Ag-nanoparticles (Figure 9), it may be due to the high repulsive and attractive force exist between nanoparticles. Similar study was found in *Gracilaria corticata,* zeta potential was -26.2 mV reported by Kumar et al. (2013) [12].

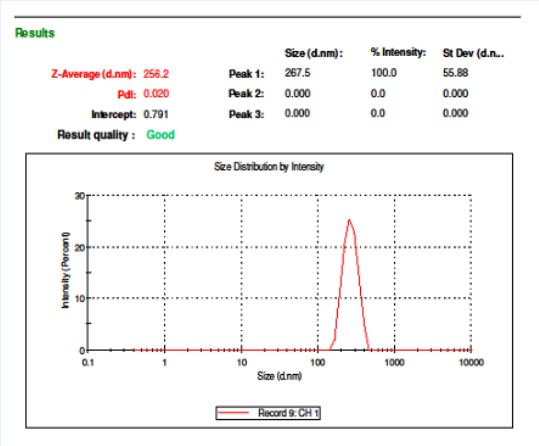


Figure 8: Showing size distribution of biosynthesized Silver Nanoparticles.

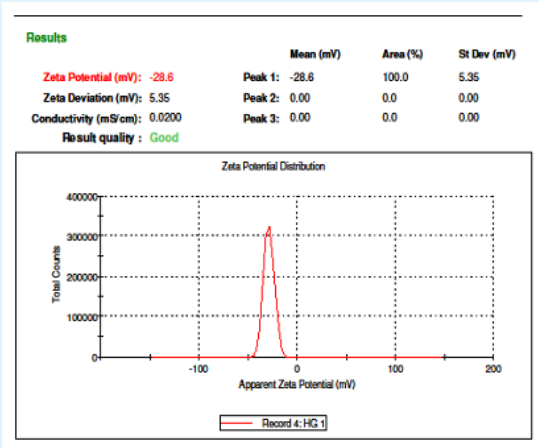


Figure 9: Showing Zeta potential of biosynthesized Silver Nanoparticles.

X-ray Diffraction Measurement

The specific nature of biosynthesized Silver Nanoparticles was analysed by XRD spectrum. The peaks produced in X-ray diffraction spectrum demonstrated the formation of spherical biosynthesized Silver Nanoparticles as the peaks have broader base and the narrower apex which indicated the presence of reduced crystal size Silver Nanoparticles. The observed peaks were found at 2θ values at 27.50, 32.25°, 47.25° and 51.25. The equation used for analysis of the grain size as: $\beta = \pi/180\times$ width (x); $D = k\lambda/\beta\cos\theta$ (nm), X= 0.21246, $2\theta = 32.50°$. The grain size (D) was 31.52 nm (Figure 9a).

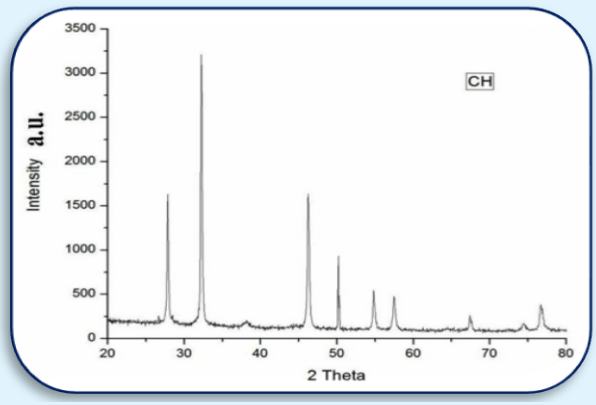


Figure 9a: Showing the XRD graph of biosynthesized Silver Nanoparticles.

Potential for Seed Germination

Experiment on seeds of *Abelmoschus esculentus*: The relative seed germination of seeds of *Abelmoschus* was maximum at 24 hours and 48 hours with the highest relative seed germination (250) and the germination index (857.14) and the next highest germination index was 845.07 at 96 hours with the highest relative root elongation (Figure 10a). The germination percentage was the highest (100 percent) after 24, 48 and 96 hours of treatment with biosynthesized Silver Nanoparticles in comparison to seeds germination with the normal water treatment as blank (Figure 10b). The mean germination rate was the maximum at 24 hours (0.17, followed by 48 (0.096) hours of treatment with biosynthesized Silver Nanoparticles in comparison to the rate of seed germination with normal water treatment (Figure 10d). The mean germination time of seeds of *Abelmoschus* was less at 24 hours (1.2), it indicates faster seed germination with treatment of biosynthesized Silver Nanoparticles, followed by 48 hours (1.4) and 96 hours (1.8) (Figure 10c).

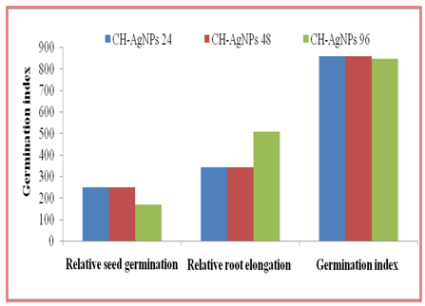


Figure 10a: Showing seed germination index.

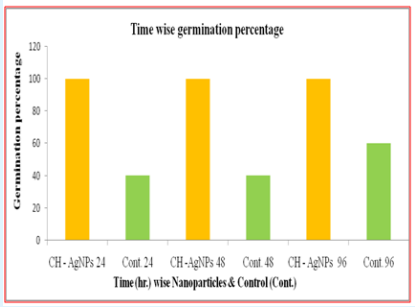
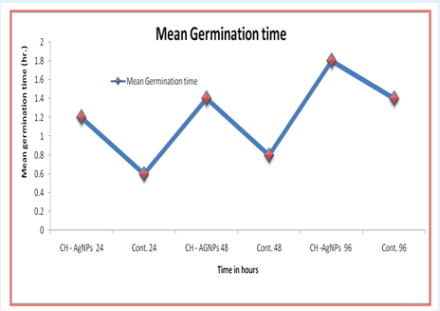
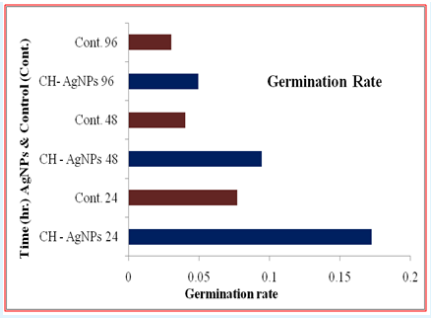


Figure 10b: Showing seed germination percentage.





Figures 10c, 10d: Showing mean seed germination time and rate. CH- (*Chlorodesmis hildebrandtii*) and Cont. - Control (Normal water treatment).

Experiment on seeds of *Raphanus sativus* var. *longipinnatus*: The seed germination index of *Raphanus* with treatment of biosynthesized Silver Nanoparticles, was maximum at 24 hours (93.33) and the relative seed germination was maximum at 48 hours (200) (Figure 11c). The seed germination percentage was higher for the seeds treated with Silver Nanoparticles for 24 hours, 48 hours and 96 hours in comparison to normal water treatment. The seed germination was 80% at 24 hours of treatment with biosynthesized Silver Nanoparticles and 100% at 96 hours and 48 hours (Figure 11d). The germination rate was maximum at 24 hours (0.14) of treatment of seeds with biosynthesized Silver Nanoparticles, followed by 48 hours of treatment (0.11) and 96 hours in compare to seed germination rate of seeds treated with normal water (Figure 11e). The mean germination time was less at 24 hours of treatment, in compare to 48 hours and 96 hours of treatment with biosynthesized Silver Nanoparticles (Figure 11f).

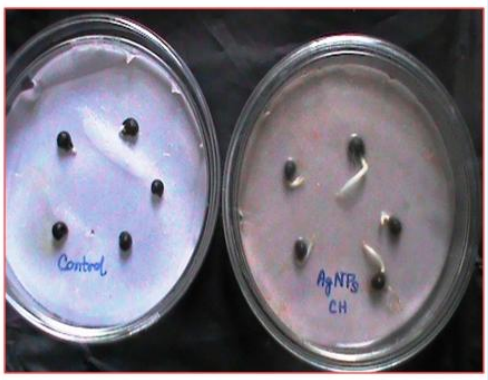


Figure 11a: Showing seed germination of Ladies finger.

Figure 11b: Showing seed germination of Radish.

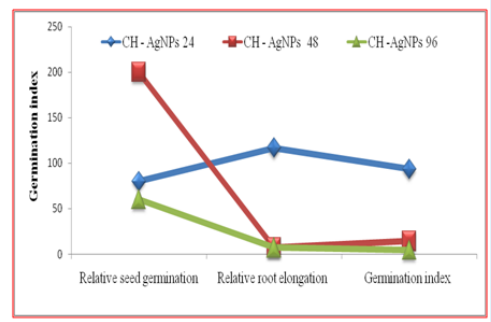


Figure 11c: Showing seed germination index.

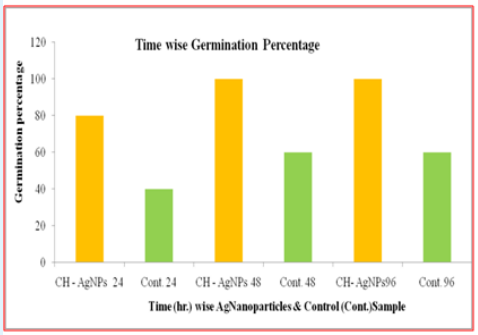


Figure 11d: Showing seed germination percentage.

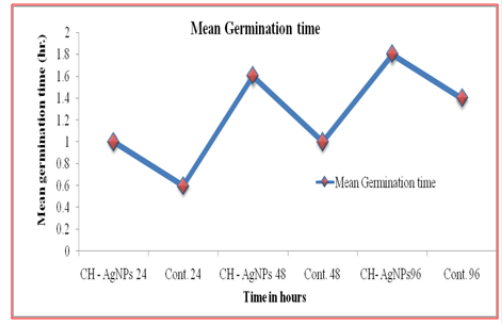


Figure 11e: Showing mean seed germination time.

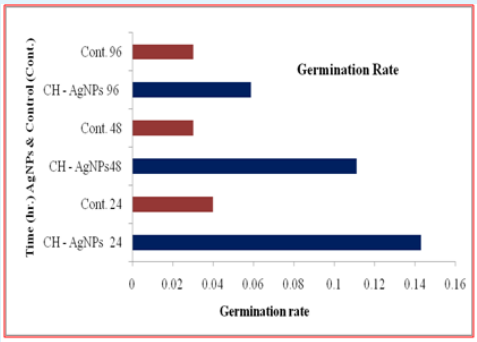


Figure 11f: Showing seed germination rate.

Experiment on seeds with normal water, seaweed extract and seaweed liquid fertilizer: For the comparative evaluation of seed germination, seeds were also treated with seaweed extract and seaweed liquid extract along with the normal water as blank and the biosynthesized Silver Nanoparticles. In case of ladies finger, no seed germination occurred with the treatment of seaweed extract and seaweed liquid fertilizer and 1mM $AgNO_3$, but the seeds treated with normal water had 60% germination and seeds treated with biosynthesized Silver Nanoparticles had 100% germination. In case of radish seeds, the highest seed germination occurred for the seeds treated with biosynthesized Silver Nanoparticles which was 100%, followed by seeds treated with normal water and seaweed extract, the germination was 60% but seed germination was 40% in case of seeds treated with seaweed liquid fertilizer and no seed germination occurred in case seeds treated with 1 mM $AgNO_3$. From, this comparative study, it was concluded that the biosynthesized Silver Nanoparticles had best promoting and boosting effect on seed germination of both species rather than normal water or seaweed extract or seaweed liquid fertilizer (Figures 12a & 12b).

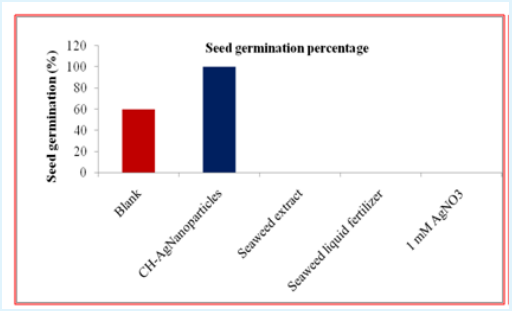


Figure 12a: Showing Ladies finger seed germination percentage

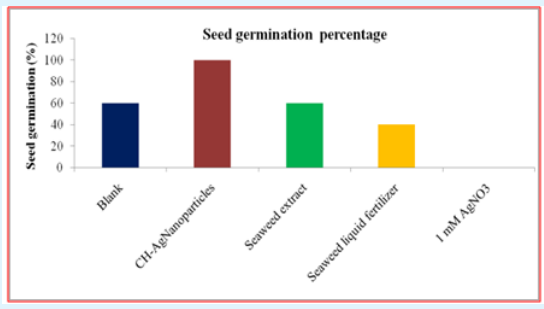


Figure 12b: Showing Radish seed germination percentage.

Antibacterial Activity

The antibacterial activity of biosynthesized Silver Nanoparticles was assayed for six human pathogenic bacteria such as Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterococcus faecalis, and Proteus mirabilis, the different strain of which had been reported for multidrug resistant properties. The highest inhibitory zone was found against Klebsiella pneumoniae, the zone of inhibition was 1.5±0.5cm, the next highest zone of inhibition produced against Escherichia coli (0.63±0.05cm), followed by Proteus mirabilis (0.23±0.05cm), in comparison to the zone of inhibition of antibiotic Chloramphenicol (5mg/ml) and $AgNO_3$ (1mM) as negative control. The inhibitory activity of $AgNO_3$ (1mM) was less for all six pathogens in comparing to seaweed synthesized Silver Nanoparticles and the antibiotics. But the inhibitory effect of seaweed synthesized Silver Nanoparticles was very less for Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus faecalis in comparing to antibiotic Chloramphenicol (5mg/ml) and the negative control $AgNO_3$ (1mM) (Figure 13).

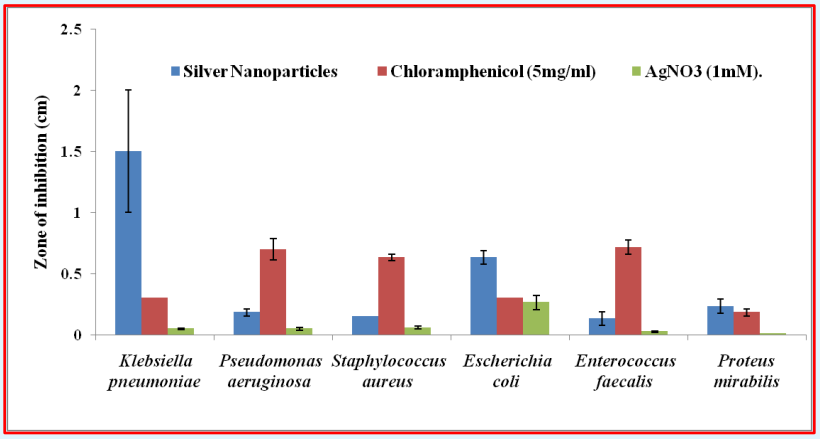


Figure 13: Showing the antibacterial activity of biosynthesized Silver Nanoparticles.

Conclusions

Current study attempted the biosynthesis of Silver Nanoparticles by using five seaweeds such *Chlorodesmis hildebrandtii.* The biosynthesized Silver Nanoparticles from *Chlorodesmis hildebrandtii* showed 100% seed germination for both ladies finger and radish seeds. The time dependent treatment of seeds by biosynthesized Silver Nanoparticles showed that increased of duration of treatment of seeds with biosynthesized Silver Nanoparticles had promoting effect of seed germination. The seeds germination ranged from 20-60% for both the seeds treated with normal water as control. For seaweed extract and seaweeds liquid bio fertilizer treatment, the seeds germination ranged from 20-60% for both seeds. It can be concluded that biosynthesized Silver Nanoparticles from *Chlorodesmis hildebrandtii* had the best positive promoting effect on seed germination of both studied seeds. So, after further investigation of the effect of this biosynthesized Silver Nanoparticles on fruiting and whole life cycle of these two vegetables plants, it will be confirmed to use the biosynthesized Silver Nanoparticles as bio-nano-fertilizer. There are a few of literatures available regarding the application of biosynthesized Silver Nanoparticles as bio-nano-fertilizer. So, this current study will be initiatory approach to develop the nano-bio-fertilizer form biosynthesized Silver Nanoparticles from seaweed. This work is first approach on the study of the potentiality of these particular seaweed synthesized Silver Nanoparticles for seed germination and seedling growth. The bio-nano-fertilizer will be applicable to the mass scale field cultivation of crop and vegetables production after its trial with whole life cycle of the vegetable plants or crop plants.

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Conflict of Interest: There are no conflicts of interest to be declared.

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