

Biosynthesis of Silver Nanoparticles by *Chaetomorpha antennina* (Bory de Saint-Vincent) Kutzing with Its Antibacterial Activity and Ecological Implication

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Abstract

The aqueous extract of green seaweed, *Chaetomorpha antennina* was used for the biosynthesis of Silver Nanoparticles. The Silver Nanoparticles were formed due to the reduction of silver nitrate to aqueous silver ions in the presence of seaweed extract. The synthesized silver Nanoparticles were characterized by UV-Visible Spectrophotometer, Fourier Transform Infrared (FT-IR) Spectroscopy, Scanning Electron Microscopy and Dynamic Light Scattering (DLS). The biosynthesized Silver Nanoparticles were assayed for its antibacterial activity which recorded that it had the highest activity against *Escherichia coli*, the zone of inhibition was 1.16 ± 0.00 cm, the next highest inhibitory activity was found against *Pseudomonas aeruginosa* (0.81 ± 0.06 cm) and also quite good zone of inhibition was found against *Klebsiella pneumoniae* (0.44 ± 0.07 cm), and *Proteus mirabilis* (0.2 ± 0.06 cm), in comparison to the positive control of Chloramphenicol (5 mg/ml) and 1 mM AgNO_3 solution as negative control. The Phyto-toxicity of biosynthesized Silver Nanoparticles were analysed by evaluating its effects on seed germination of *Abelmoschus esculentus* and *Raphanus sativus* var. *longinnatus* seeds which showed that biosynthesized Silver Nanoparticles had excellent promoting effect on seed germination and the seedling growth on both seeds.

Keywords: Seaweed; Green synthesis; Silver nanoparticles; Antibacterial activity; Seed germination

Introduction

Nanoparticles are peculiar in their physical and chemical properties in compare to bulk compounds. So, Nano scale research gain the attention in the modern field of material science and research groups. In various field, the novel application of Nanoparticles are gradually improving and became as emerging needs. But chemical originated silver Nanoparticles had been reported for its toxic nature to vegetation. So, to know the toxic level and mechanism, chemical originated silver Nanoparticles had been investigated for its effect on various seed germination. It had been reported that chemical Silver Nanoparticles and powder Silver Nitrate (AgNO_3) solution had significant reducing and toxic effect on seed germination of Radish (*Raphanus Sativus* L.) [1]. The effect of chemical Fe and Ag Nanoparticles on seed germination had been evaluated for flax (*Linum usitatissimum* L., cv. Electra), ryegrass (*Lolium perenne* L.), and two-rowed barley (*Hordeum vulgare* L.) in combination of soil compositions [2] and the effect of Ag-Nanoparticles also had been reported to be evaluated for its effect on seed germination of *Vigna radiata* [3]. The effect of chemical originated nano silver on seed germination and seedling growth in fenugreek seeds was investigated by some researcher [4]. The majority of previous reports showed that chemical originated Ag-Nanoparticles had toxic effect on seed germination and vegetation. So, eco-friendly and Phyto-friendly bio-originated silver Nanoparticles may negotiate the toxic effect of chemical originated Silver Nanoparticles. Some seaweeds had been reported as raw material for green synthesis of Silver Nanoparticles such as *Caulerpa racemosa* [5] *Turbinaria conoides* [6] and some seaweed synthesized silver Nanoparticles were evaluated for their potential for seed germination [7-12] which reported that seaweed synthesized Silver Nanoparticles was Phyto-friendly and had promoting effect on seed germination but it was promoting the seeds germination in compare to normal water and also seaweed extract and seaweed liquid bio-fertilizer. Silver Nanoparticles was also reported for their application in medicine to reduce the infection in burn treatment and prevent the adherence of dental bacterial colonization [13,14]. Some seaweed such as *Padina tetrastromatica* [15] and *Turbinaria*

conoides [16] synthesized Silver Nanoparticles had been reported for their antibacterial activity. The effects of chemical Nanoparticles on environment is not yet completely known to us, but some work had been reported the toxic effects of metal Nanoparticles to the environment both aquatic and terrestrial as well as human health [17,18]. So, eco-friendly biosynthesized Silver Nanoparticles are now leading interest for bio-safety purpose.

Materials and Methods

Synthesis of silver nanoparticles

Seaweed extract preparation: The fresh seaweed had been collected from Olaikuda ($09^{\circ}18'.390''\text{N}$ and $079^{\circ}20'.076''\text{E}$), Rameshwaram, Southeast coast of India. Seaweed was identified with standard taxonomic key of CMFRI. It was washed with *in-situ* sea water and distilled water thrice. Then, 20 g 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 what man No. 1 filter paper and repeatedly filtered with thin layer of cotton to get clear seaweed extract. The crude seaweed extract was stored in 4°C for further use (Figure 1).

Synthesis of Ag-nanoparticles

The aqueous 1 mM Silver Nitrate (AgNO_3) solution was prepared with

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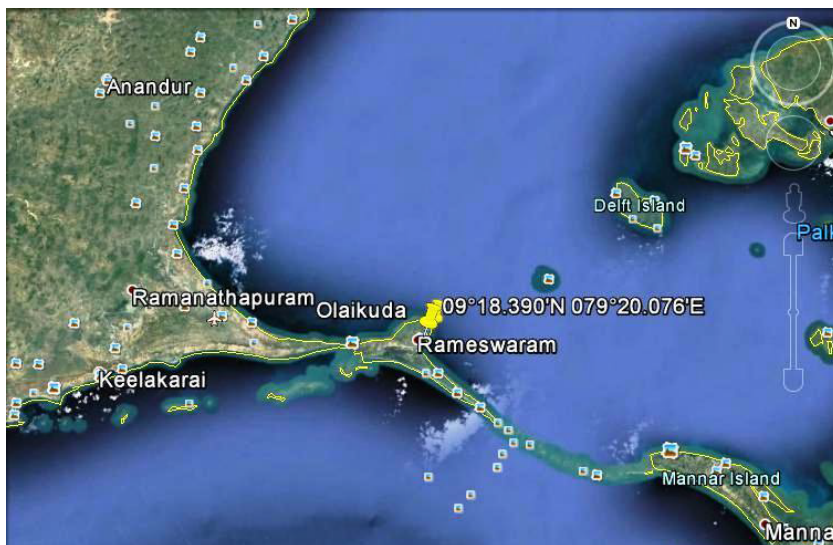


Figure 1: Map showed the sampling location.

Silver nitrate powder. For typical biosynthesis 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 rpm. The colour change of solution indicated the formation of silver Nanoparticles.

Characterization of Ag-nanoparticles

UV-visible spectrophotometer: After 72 hours of biosynthesis of Silver Nanoparticles particles, for confirmation of bio-synthesis and characterization, the solution was scanned (300-700 nm) with UV-Vis Spectrophotometer (UV-2600 SHIMADZU).

Fourier transform infrared (FT-IR) spectroscopy: After biosynthesis of Silver Nanoparticles, the solution had been centrifuged at 5000 rpm 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.

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.

Antimicrobial activity

Antibacterial activity of biosynthesized Silver Nanoparticles using aqueous extract of *Chaetomorpha antennina* was assayed by agar disc diffusion method against six human pathogenic bacteria such as *Klebsiella pneumoniae*, *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 Silver Nitrate (AgNO_3) solution was used as negative control. The 20 μl of biosynthesized Silver Nanoparticles solution, antibiotic solution (positive control) and Silver Nitrate solution (negative 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.

Seed germination test

The seeds of *Abelmoschus esculentus* (L.) Moench (Family - Malvaceae) and *Raphanus sativus var. longinatus* L. H. Bailey (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 the following equations [19-22].

$$\text{Germination Percentage (GP \%)} = \left(\frac{Gf}{n}\right) \times 100 \quad (1)$$

where Gf is the total number of germinated seeds at the end of experiment and n is the total number of seed used in the test.

$$\text{Mean Germination Time (MGT)} = \frac{\sum NiDi}{n} \quad (2)$$

where Ni is number of germinated seeds until the ith day and Di is number of days from the start of experiment until the ith counting and n is the total number of germinated seeds.

$$\text{Germination Rate (GR)} = \frac{\sum Ni}{\sum Ti Ni} \quad (3)$$

where Ni is the number of newly germinated seeds at time Ti.

$$\text{GR} = \frac{a}{1} + \frac{b-a}{2} + \frac{c-b}{3} + \dots + \frac{n-n-1}{N} \quad (4)$$

Relative root elongation (E)=(Mean root length with NPs)/(Mean root length with control) × 100

Germination index (GI)=(Relative seed germination) × (Relative root elongation)/100

Where,

Relative seed germination=(Seeds germinated with NPs)/(Seeds germinated with control) × 100.

Statistical analysis

The zone of inhibition of antibacterial activity of biosynthesized Silver Nanoparticles, solution of antibiotic and Silver Nitrate (AgNO₃) 1 mM solution were measured in triplicates and expressed as mean and standard deviations.

Results and Discussions

Synthesis of silver nanoparticles

The mixing of seaweed aqueous solution with Silver Nitrate (1 mM) produced reddish brown 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⁺ (Figures 2 and 3). Due to the surface Plasmon vibrations among the produced silver Nanoparticles, the colour change occurred [23].

Characterization of synthesized nanoparticles

UV-visible spectrophotometer: UV-Visible Spectroscopic absorbance



Figure 2: *Chaetomorpha antennina* (Division - Chlorophyta, Class - Chlorophyceae, and Family - Cladophoraceae).



Figure 3: Showed the synthesis of Nanoparticles. (a) - 1 mM Silver Nitrate solution, (b) - Seaweed extract, (c) - Biosynthesized Silver Nanoparticles.

peak at 435.5 nm (OD-0.643) *Chaetomorpha antennina* indicated the synthesis of Silver Nanoparticles (Figure 4).

Fourier transform infrared (FT-IR) spectroscopy: The biosynthesized Silver Nanoparticles were analysed for identification functional groups with the help of stretches and bend found in IR spectra due to vibrations for the presence of various functional groups of various compounds. The major peak was observed in the IR spectra of biosynthesized Silver Nanoparticles at 3449.61 cm⁻¹ indicated the presence of O-H stretch, H-bond, amine and amide group (N-H) which may be helping to capping and stabilizing the Nanoparticles and the same major peak at 3453.31 cm⁻¹ was also found for aqueous seaweed extract which also indicated the presence of O-H stretch, H-bond, amine and amide group (N-H). The stretches at 2922.23 cm⁻¹ and 2847.27 cm⁻¹ were developed due to the presence of alkenes (C-H) group, acid (O-H) group and aldehydes group. Some peaks at 1633.76, 1384.58 and 1017.90 cm⁻¹ appearing for Alkenes (C=C), Amide (N-H) groups, Nitro (N-O), ether and ester groups. The variation and shifting of peaks in the IR spectra of biosynthesized Silver Nanoparticles attributed the reduction of silver ions into their respective Nanoparticles as shown in Figures 5 and 6. The peak at 1636.69 cm⁻¹ stretch also present in the IR spectra of aqueous extract of seaweed which appearing to have the presence of alkenes (C=C), amide (N-H) groups. The peak at 622.01 cm⁻¹ may be assigned due to the stretching of aromatic rings. The comparison of IR spectra of normal seaweed extract and the biosynthesized Silver Nanoparticles, clearly explained that the more functional groups were present in the biosynthesized Silver Nanoparticles, may be due to which the Silver Nanoparticles had the promoting effect on seed germination and they also had inhibitory activity against the pathogen.

Scanning electron microscopy: The SEM image (Figure 7) represented the morphological characterization of biosynthesized Silver Nanoparticles. It showed the presence of high density, hexagonal, cubical and well distributed biosynthesized Silver Nanoparticles synthesized from aqueous extract of *Chaetomorpha antennina*.

Dynamic light scattering (DLS): The Z-average size distribution

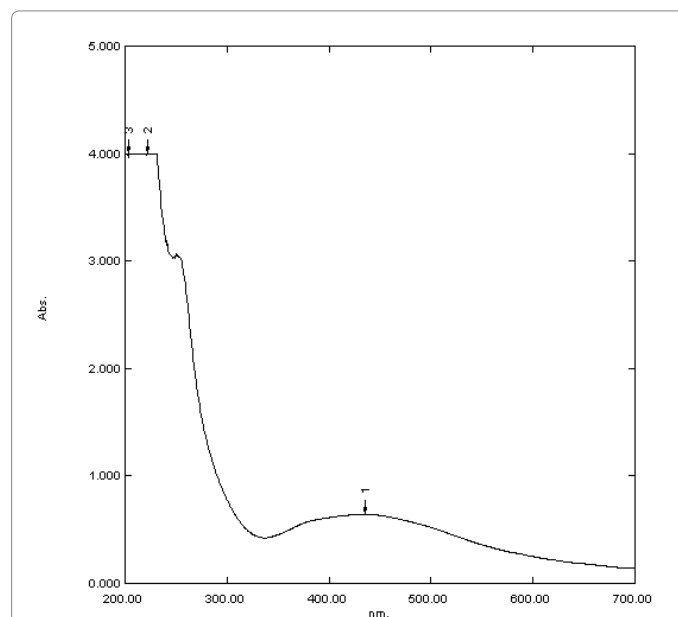


Figure 4: UV-Vis Spectra of *Chaetomorpha antennina* synthesized Ag-Nanoparticles.

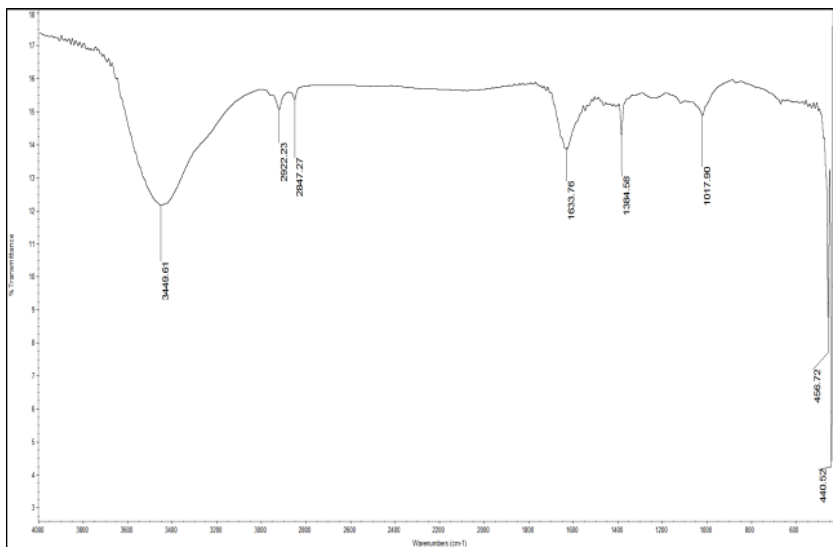


Figure 5: FT-IR spectra of aqueous extract of *Chaetomorpha antennina* extract.

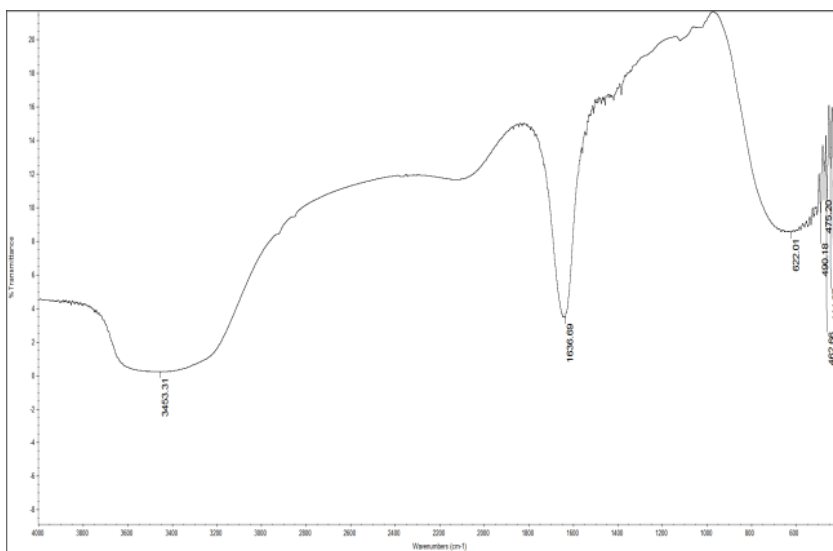


Figure 6: FT-IR spectra of Silver Nanoparticles synthesized by *Chaetomorpha antennina* extract.

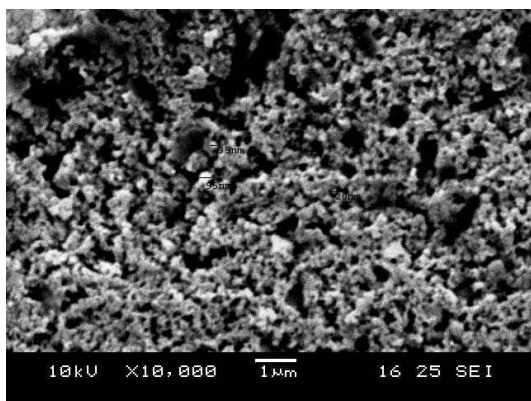


Figure 7: SEM image of biosynthesized silver nanoparticles.

of Ag-Nanoparticles were found 256.2 d.nm and particles were well distributed in the water solution (Figure 8). The zeta potential value of Ag Nanoparticles, -31.4 mV, indicated the high stability of Ag-Nanoparticles (Figure 9), it may be due to the high repulsive and attractive force exist between Nanoparticles [24] The similar study had been reported for biosynthesized Silver Nanoparticles by *Gracilaria corticata*, zeta potential was -26.2 mV [25].

The seeds treated with biosynthesized Silver Nanoparticles, had the highest germination index at 48 hours (germination index-551.40), with the highest relative root elongation and relative seed germination, followed by a germination index at 96 hours (germination index-216.72) (Figure 10a). The germination percentage was 80% at 48 hours and 96 hours in case of seeds treated with biosynthesized Silver Nanoparticles (CA-AgNPs) in comparison to the seeds treated with normal water, the germination percentage was 40% and 60% (Figure 10b). The mean germination time was less at 24 hours (0.6 h), but it was gradually

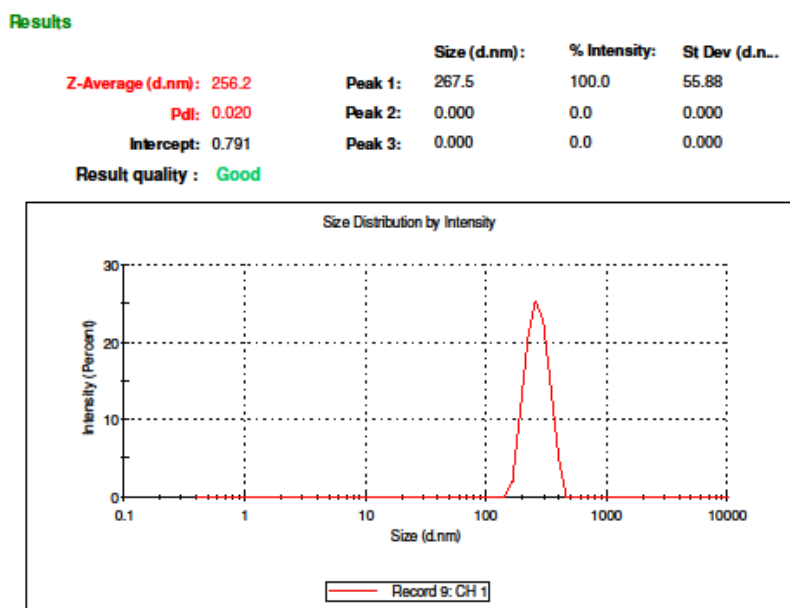


Figure 8: The size distribution of biosynthesized silver nanoparticles.

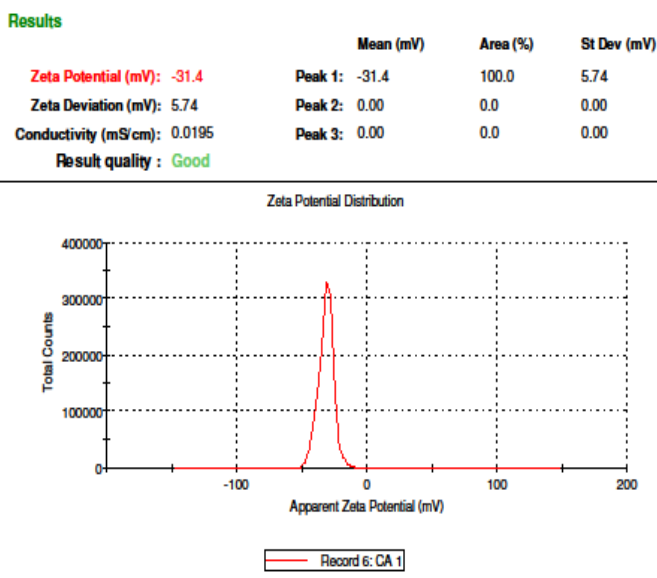


Figure 9: The Zeta potential of biosynthesized Silver Nanoparticles.

increased with the duration of treatment and reached the maximum at 96 hours, but mean germination time was more in comparison to control (Figure 10c). The germination rate was the highest at 24 hours of treatment, and gradually decreased with duration of treatment and germination rate was lower for the seeds treated with biosynthesized Silver Nanoparticles for 96 hours in comparison to normal water treated seeds (Figure 10d).

Experiment on seeds of *Raphanus sativus* var. *longinnatus*: The germination index for biosynthesized Silver Nanoparticles treated seeds were the highest at 96 hours (germination index-83.52) with maximum relative seed germination (200). At 24 hours of treatment,

the germination index was medium (66.66), but at 48 hours, it was less (50.87) (Figure 11a). The mean seed germination percentage was the maximum at 96 hours treatment with biosynthesized Silver Nanoparticles. The germination percentage was comparatively higher for seeds treated with biosynthesized Silver Nanoparticles at 48 hours (62.5%) and 96 hours (75%) in comparison to normal water treated seeds (40%) and (60%) (Figure 11b). The mean seed germination time was less at 24 hours, which explained that germination was faster at the beginning, but the mean seed germination time was gradually decreased with increase of time of treatment with biosynthesized Silver Nanoparticles and normal water. But the mean seed germination time

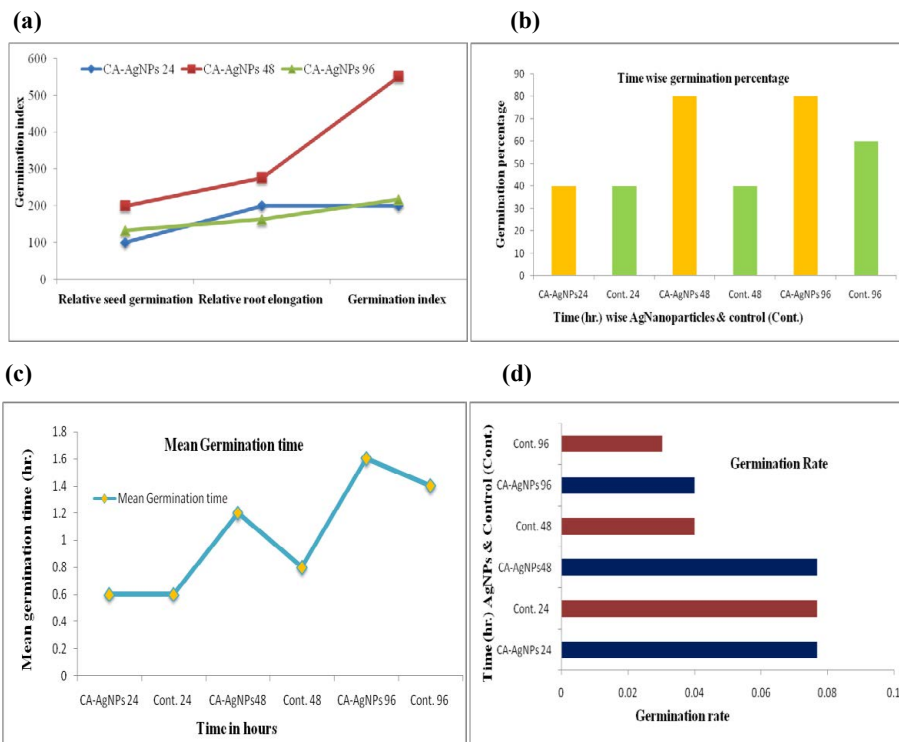


Figure 10: (a) Seed germination index (b) Seed germination percentage (c) Mean seed germination time (d) Seed Germination rate.

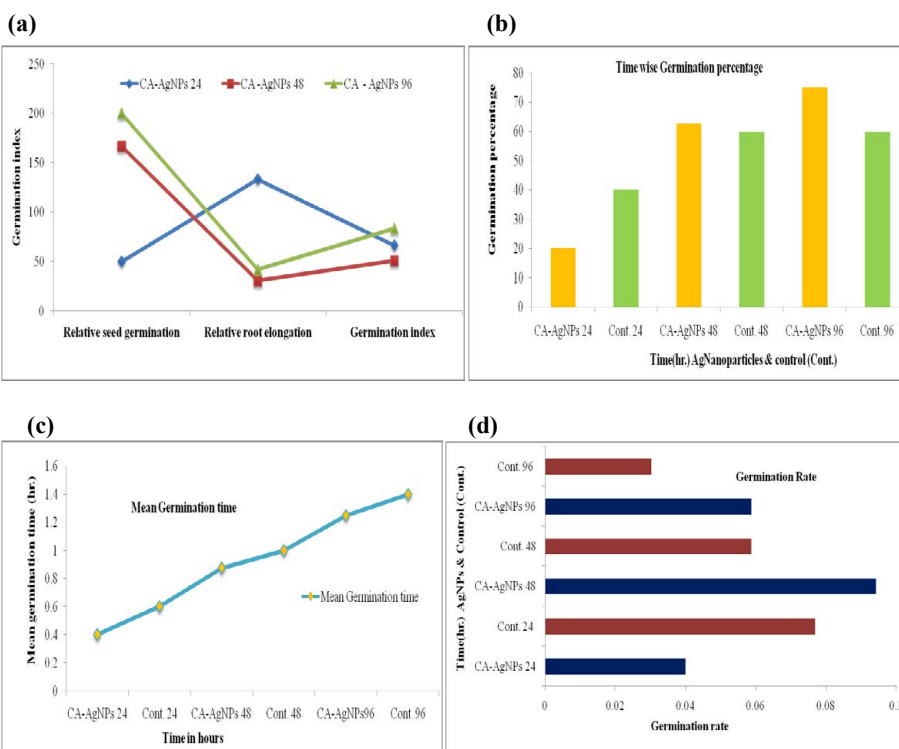


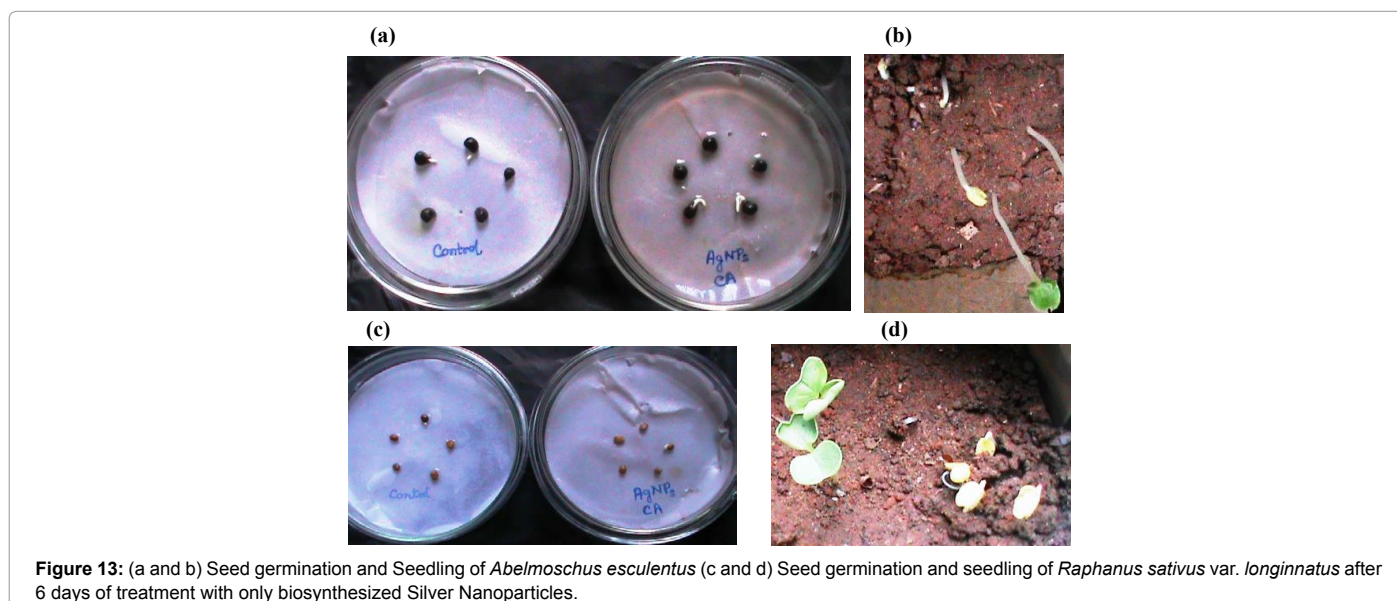
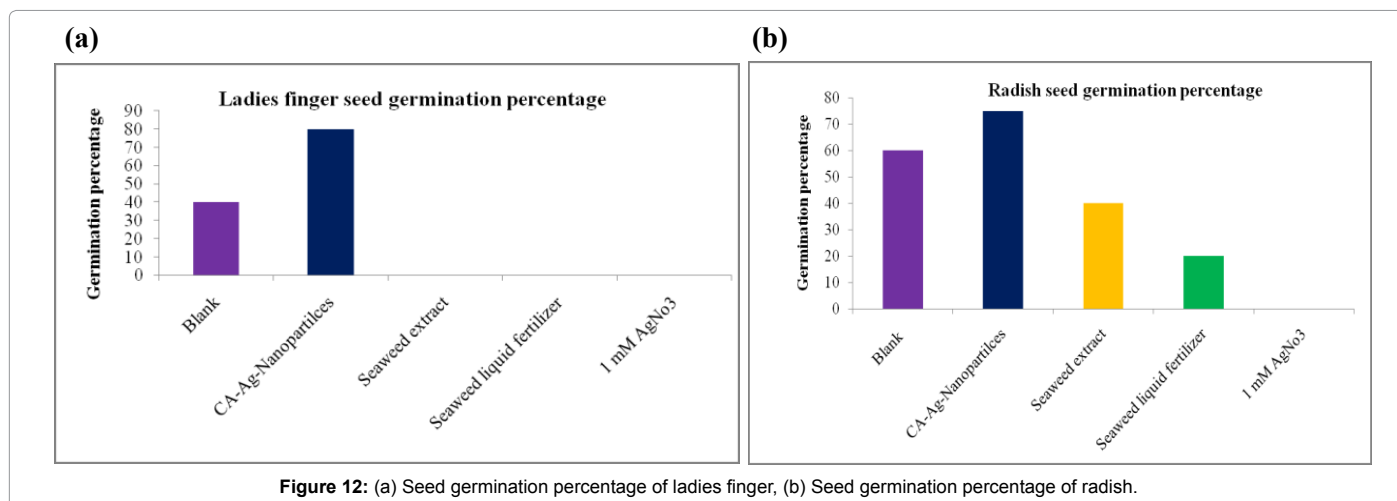
Figure 11: (a) Seed germination index (b) Seed germination percentage (c) Mean seed germination time (d) Seed germination rate.

of biosynthesized Silver Nanoparticles treated seeds were always lower than normal water treated seeds (Figure 11c). The seed germination rate was the highest at 48 hours of treatment of seeds with biosynthesized Silver Nanoparticles in comparison to normal water treated seeds and the seed germination rate was higher for biosynthesized Silver Nanoparticles treated seeds at 96 hours. The time wise change of germination rate, mean germination time and germination index may be due to the easy penetration of biosynthesized Silver Nanoparticles and its faster circulation due its nano-sized (Figure 11d).

Experiment of seed germination with Silver nitrate solution, seaweed extract and seaweed liquid bio fertilizer: The silver nitrate (AgNO_3) 1 mM solution was used as a negative control to test its effect on seed germination in comparison to normal water, for both seeds radish and ladies finger. The ladies finger, seed germination was 40% and the radish seed germination was 60% with treatment of normal water, but no seed germination occurred for seeds treated with 1 mM silver nitrate solution. It proved that 1 mM AgNO_3 solution was toxic to both *Abelmoschus* and *Raphanus* seed germination. Both seeds were also treated with normal seaweed aqueous extract and seaweed liquid fertilizer of *Chaetomorpha antennina* for one week, but no seed

germination occurred in the case of the seeds treated with seaweed extract and seaweed liquid fertilizer for *Abelmoschus* but for *Raphanus*, seed germination was 40% with the treatment of the seaweed aqueous extract and 20% seeds were germinated with treatment of seaweed liquid bio-fertilizer (Figure 12a and 12b). The results concluded that the biosynthesized Silver Nanoparticles was the best promoter for seed germination and seedling growth in comparing to normal water, seaweed extract and seaweed liquid fertilizer (Figure 13a-13d). The seedling growth was also best in case of seeds treated with biosynthesized Silver Nanoparticles. It had been previously reported that seeds treated with biosynthesized Silver Nanoparticles synthesized by *Sargassum cinctum*, in comparing to seeds treated with normal water treatment as control had the better effect on *Abelmoschus esculentus* seed germination and the seedling growth. So, biosynthesized Silver Nanoparticles had been proved as an excellent bio fertilizer for promoting seed germination and seedling growth of *Abelmoschus esculentus* [7].

Antibacterial activity of seaweed synthesized silver nanoparticles: The antibacterial activity of biosynthesized Silver Nanoparticles was assayed against six pathogenic bacteria. It had been reported that biosynthesized Silver Nanoparticles had the maximum inhibition



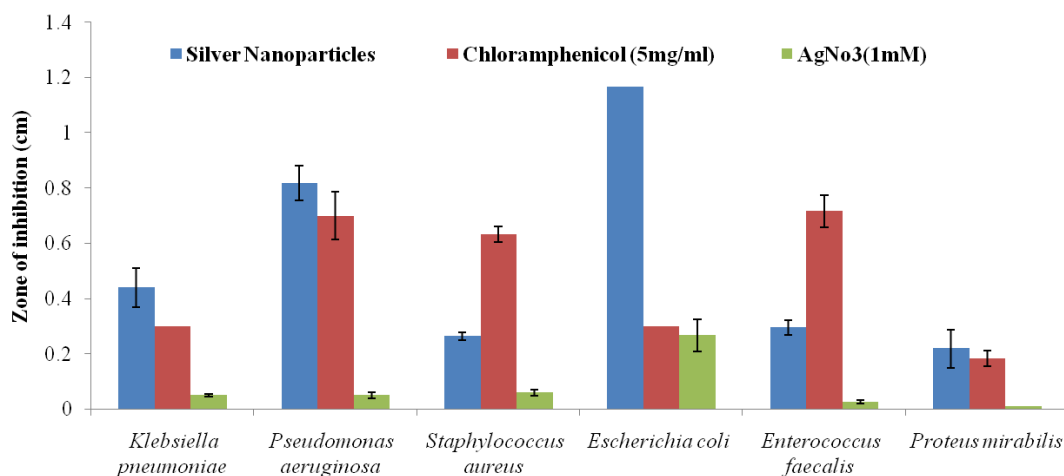


Figure 14: The zone of inhibition by Silver Nanoparticles, antibiotic and silver nitrate solution.

activity against *Escherichia coli*, the zone of inhibition was 1.16 ± 0.00 cm, the next highest inhibitory activity was found against *Pseudomonas aeruginosa* (0.81 ± 0.06 cm) and also quite good zone of inhibition was found against *Klebsiella pneumoniae* (0.44 ± 0.07 cm), and *Proteus mirabilis* (0.2 ± 0.06 cm), in comparison to the positive control of Chloramphenicol (5 mg/ml) and 1 mM silver nitrate (AgNO_3) solution was used as negative control which showed very less antibacterial activity against six pathogens. The biosynthesized Silver Nanoparticles had comparatively less inhibition activity against *Staphylococcus aureus* and *Enterococcus faecalis* in comparison of positive control Chloramphenicol (5 mg/ml) (Figure 14).

Conclusions

The effect of biologically synthesized Silver Nanoparticles from *Sargassum plagiophyllum* on seed germination of *Arachis hypogaea*, *Vigna mungo*, and *Vigna radiata* had been reported that biosynthesized Silver Nanoparticles had growth promoting activity in plants and non-toxic to the plants and also supported the growth of plants even in the absence of growth hormones [26]. This is the first time report on synthesis of Silver Nanoparticles using *Chaetomorpha antennina* and also its effect on seed germination and antibacterial activity. In our study, the seeds were treated only with biosynthesized Silver Nanoparticles for germination and the growth of seedling of both plants was for one week. The both results showed that biosynthesized Silver Nanoparticles had promoting effect on seed germination and seedling growth. The seed germination percentage, seed germination rate and seedling growth were the highest in the case of seeds treated with biosynthesized Silver Nanoparticles may be due to the presence of Amine, Amide and Nitro groups which promoted the germination rate, germination mean time and the seedling growth and it may be due to its nutritious compositions. The seed germination of the two species was excellent with biosynthesized Silver Nanoparticles treatment, but the extensive and elaborate study on the effect of biosynthesized Silver Nanoparticles on both plants complete life cycle and the yield production will be helpful for future use of biosynthesized Silver Nanoparticles as nano-bio-fertilizer.

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Conflict of Interest

There are no conflicts of interest to be declared.

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