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Green Synthesis of Silver Nanoparticles by Sargassum Cinctum J. Agardh and their Potential for Seed Germination

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ABSTRACT

Nanoparticles are recognizing for their applications in various fields of medical sciences and they are also under safety concern. The effects of metals nanoparticles on environment is not yet completely known to us, but some of the study had been elaborated the toxic effects of metal nanoparticles to the environment both aquatic and terrestrial as well as human healths. So, eco-friendly green synthesized nanoparticles are now leading our interest for bio-safety purpose. The aqueous solution of brown seaweed (*Sargassum cinctum)* had been utilized for green synthesized 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 using UV-Visible Spectrophotometer, FTIR and Scanning Electron Microscopy. The biochemical compositions such as carbohydrates, proteins, lipids and starch were estimated for the mentioned species according to standard methods and the total phenol, in-vitro antioxidant activity and free radicals DPPH scavenging activities were also estimated. The phyto-toxicity of green synthesized silver nanoparticles was evaluated by studying its effect on seed germination of *Abelmoschus esculentus*, one of the important vegetable items in Indian market.

Keywords: *Green synthesis, silver nanoparicles, seed germination*

INTRODUCTION:

Seaweeds are most promising nutritive lower group of plants with thallus structure. They are the incredible renewable marine resources. Some of the seaweeds have been reported for having high carbohydrates, protein, lipids, vitamins and minerals (Rupérez 2002; McDermid and Stuercke 2003; Ortiz et al. 2006; Marsham et al. 2007; Chakraborty and Santra 2008; Matanjun et al. 2009, Manivannan et al. 2008 & 2009 and Roy et al. 2017a & b). It is believed that nanoparticles are the agglomerates of nano sized (1-100nm) particles and primarily classified as organic (carbon nanoparticles) and inorganic (magnetic nanoparticles, noble metals nanoparticles such as silver, gold and platinum and semiconductor nanoparticles (zinc oxide & sulphide, titanium dioxide) (Williams 2008). The nanoparticles had been exposing rapidly into the environment from natural and anthropogenic activities (Klaine et al. 2008 & Farre et al. 2009), as 15 % of all product in global market (e.g. electronics, engineering, medicine, wound dressing, socks and other textiles, air filter, tooth paste), incorporated with nanoparticles for their manufacturing (Federici et al. 2007 & Dawson et al. 2008). So, focusing on environmental safety and to reduce toxicity of metals nanoparticles, eco-friendly

nanoparticles had been synthesized using various biological sources. Some of the seaweeds had been reported for its mediated nanoparticles synthesis and had been reported for their various medical applications especially antibacterial activity (Shanmugam et al. 2014; Venkatpurwar and Pokharkar, 2011); and water filters (Jain and Pradeep, 2005), bio sensors (Chen et al. 2007), in controlling plant pathogens (Krishnaraj et al. 2012) and anti-fungal activity (Devi et al. 2014; Kumar et al. 2013). Recently, seaweeds liquid bio-fertilizer has been reported for promoting the growth of various cereals crops and vegetables (Hernández-Herrera et al. 2013; Safinaz et al. 2013; & Soad et al. 2016). In this present work, the seaweed synthesized extracellular eco-friendly silver nanoparticles have been evaluated for its photo-toxicity for studying their effect on seed germination including the analysis of its biochemical compositions, total phenol and in-vitro- antioxidant activities. The aim of this study is to analyse the properties of seaweeds mediated silver nanoparticles as nano bio-fertilizer.

MATERIALS AND METHODS:

Synthesis of Silver Nanoparticles:

Seaweed extract preparation: The fresh seaweed had been collected from Vadakkadu (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. 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.

Fig 1: ***Sargassum cinctum***

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.

Estimation biochemical compositions: The crude carbohydrate was estimated by the standard Anthrone method (Hedge et al. 1962); crude proteins by biuret method (Goshev et al. 1979) and crude lipid estimated by (Yang et al. 2014).

Estimation of total phenolic content (Folin Ciocalteu's method): The 500 mg seaweed powder was dissolved in ethanol, methanol and acetone and kept for 24 hours and after 15 minutes centrifugation at 5000 rmp, the supernatant was collected for determination of total phenols. The 1 ml of aliquots of ethanol, methanol and acetone extracts and standard

gallic acid (10, 20, 40, 60, 80, 100 µg/ml) were positioned into the test tubes and 5ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent were mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to10 ml with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed. After incubation, absorbance was measured at 750 nm spectrophotometer using Perkin Flmer precisely Lambda 25 UV/Vis Spectrophotometer. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of seaweed was expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass (Bhalodia et al., 2011; Patel et al., 2010).

Estimation of total antioxidant activity (Phosphomolybdate assay): Total antioxidant activities of methanol, ethanol and acetone extracts were obtained by phospho-molybdate method using ascorbic acid as a standard (Lallianrawna et al. 2013). Briefly, the 7.45 ml of H_2So_4 (0.6mM solution), sodium sulphate of 0.9942 g (28mM) in addition to 1.2359 gm ammonium molybdate (4mM) mixed in 250 ml distilled water to prepare the TAC reagent. The 300 µl of seaweed extracts were mixed with 3 ml of TAC reagent. The reaction mixtures were incubated at 95°C for 90 minutes under water bath. Absorbance of all the sample mixtures was measured at 695nm against a blank on a UV-visible spectrophotometer. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram per gram of extract. A typical blank contained 1 ml of the reagent solution along with an appropriate volume of the solvent and incubated under similar conditions. The antioxidant capacity of plant extract solution was estimated using following formula: Total antioxidant capacity, TAC (%): = [(Control $_{absorbance}$ – Sample $_{absorbance}$) / (Control $_{absorbance}$)] x 100

(iii) Free Radical (DPPH-2,2-diphenyl-1-picrylhydrazyl) Scavenging Activity: The scavenging activity of methanol, ethanol and acetone extracts of seaweed were determined according to standard protocol (Surana et al. 2016). Briefly, 2.0 ml of 0.16 mM DPPH solution in methanol was added to the test tube containing 2.0 ml aliquot of sample. The mixture was vortexed for 1 minute and kept at room temperature for 30 minutes in the dark. The absorbance of all the sample solutions was measured at 517 nm. Sample$_{blank}$ and Control $_{sample}$ were performed according to the method. Scavenging activity of DPPH radical was calculated using the following equation

$$\text{Percentage inhibition} = \frac{(A_{control}-A_{sample}) \times 100}{A_{control}}$$

Where A_{sample} is the Absorbance of DPPH solution with sample, $A_{control}$ is the blank absorbance. Synthetic antioxidant Ascorbic acid was used as positive control.

Seed germination test (Raquel Barrena *et al.*, 2009):

The seeds of *Abelmoschus esculentus* (Family-Malvaceae) 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 (Singaravelu et al. 2007; Taga et al. 1984 and Thakkar et al. 2010).

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

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.

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

where Ni is number of germinated seeds until the i^{th} 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.

$$\text{Germination Rate (GR)} = \Sigma Ni/ \Sigma Ti\ Ni \quad (3)$$

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

GR = (a/1) + (b-a/2) + (c-b/3) +.....+ (n-n-1/N) (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

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 & DISCUSSIONS:

Synthesis of Silver Nanoparticles: It is well known that Ag-NPs exhibit reddish-brown in water (Damle et al. 2002). 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+ (fig 2). Due to the surface Plasmon vibrations among the produced silver nanoparticles, the color change occurred (Mulvaney et al. 1996)

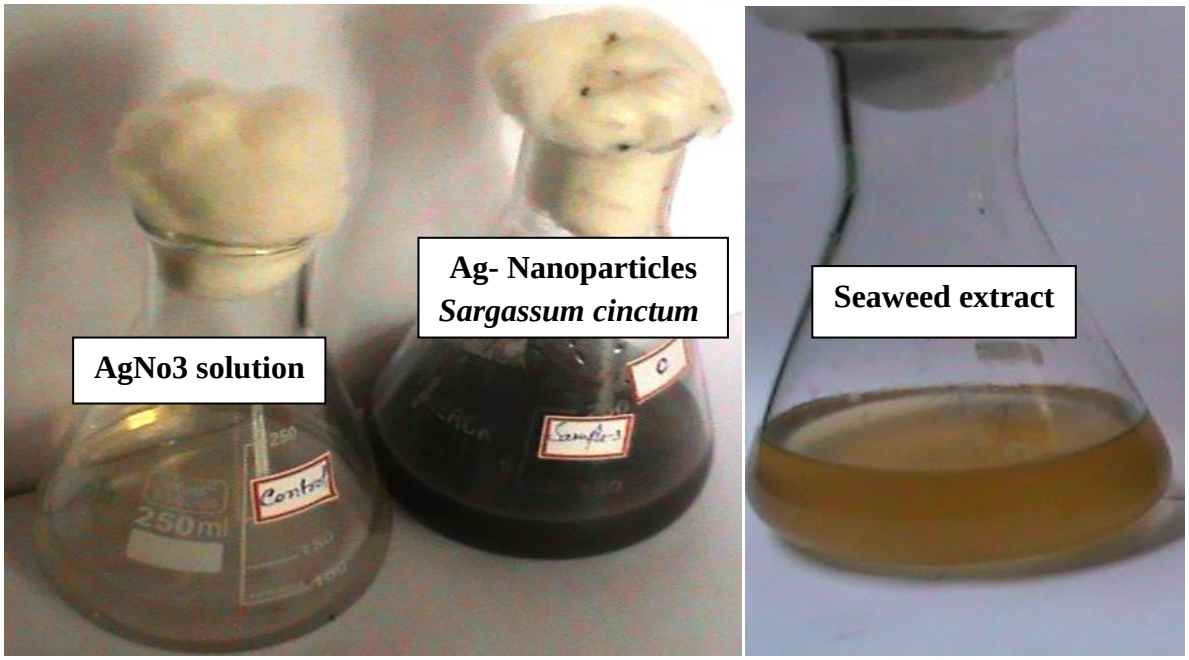

Figure 2: Showing the synthesis of nanoparticles

Characterization of synthesized nanoparticles:

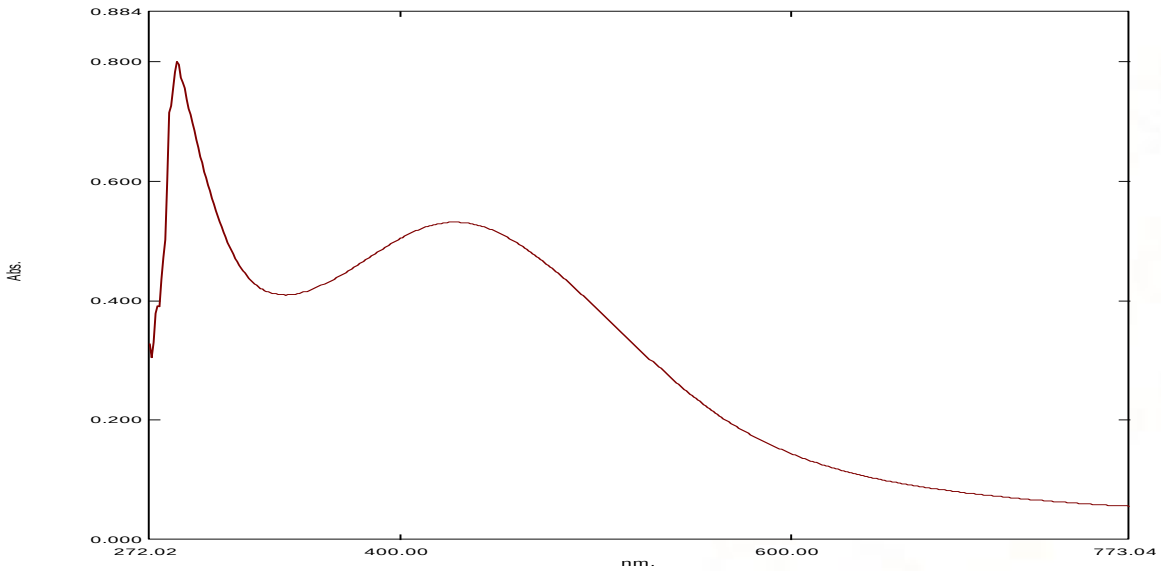

Figure 3: UV-Vis Spectra of *Sargassum cinctum* 420nm – 0.563
425nm - 0.565
430nm – 0.565
435nm – 0.564

UV-Visible Spectroscopic absorbance peak at 425 to 430 nm for *Sargassum cinctum* indicated the synthesis of silver nanoparticles.

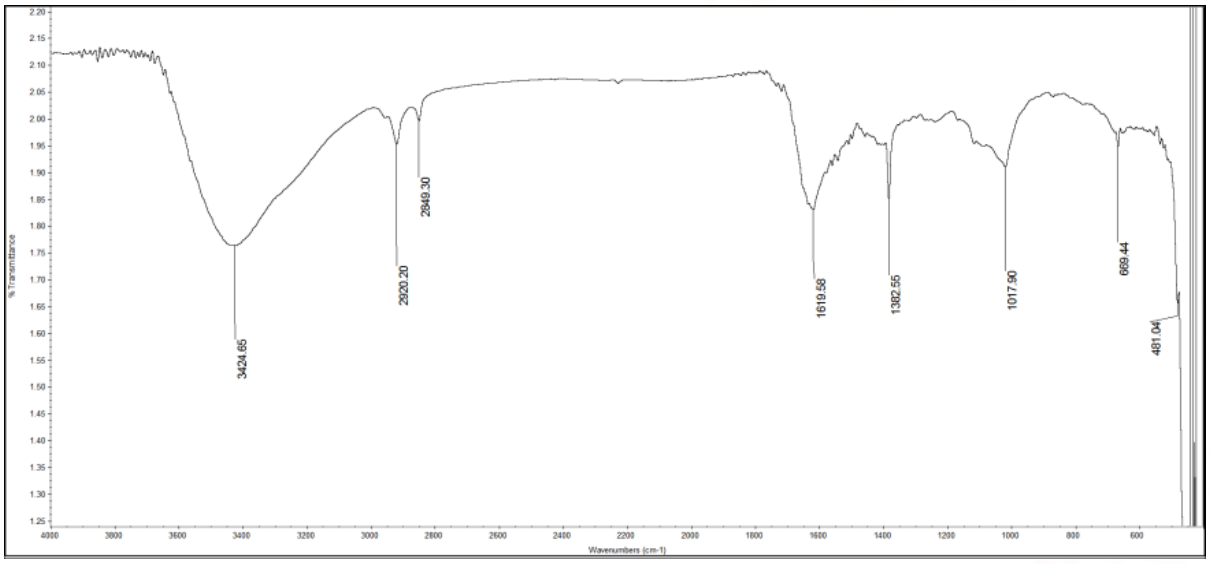

Fig 4: FT-IR spectra of silver nanoparticles synthesized by *Sargassum cinctum* extract.

The seaweed mediated silver nanoparticles were analysed for the characterization of their functional groups and their properties (table 1).

Table 1: FT-IR peak values of *Sargassum cinctum* mediated silver nanoparticles

Peaks	Bonds	Functional Groups
669.44	C-Cl, C-Br Stretch	Alkyl halide
1017.90	C-F Bend	Alkyl halide
1382.55	C-H Stretch, C-N bend	Alkane, Amine
1619.58	C=C Bending	Aromatic
2849.30	C-H Stretch	Alkyl
2920.20	O-H Stretch	Carboxylic compounds
3424.65	N-H Stretch	1˚, 2˚Amines, Amides

The results revealed from table 1 showed that the capping of ligand of the Ag-NPs may be an alkyl halide, alkanes, aromatic compound or carboxylic compound, or amide and amines

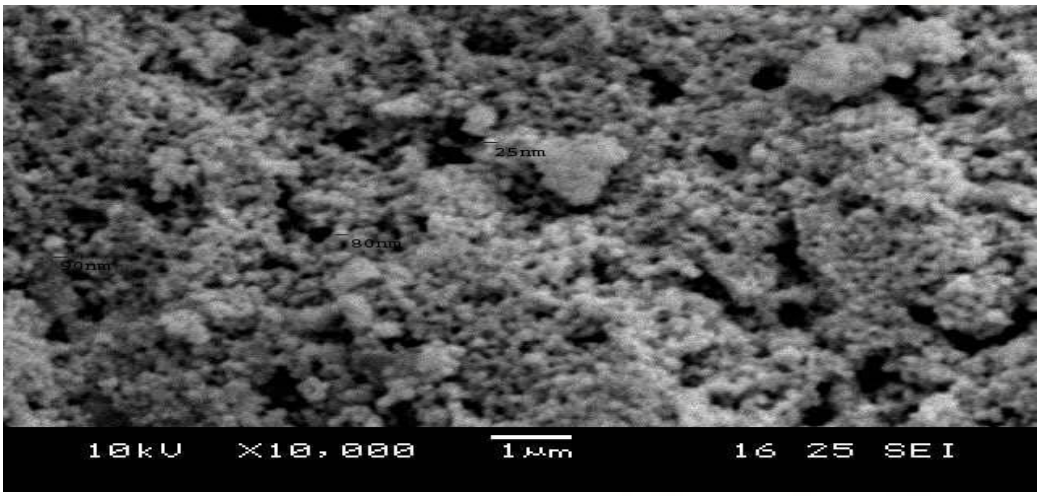

Fig 5. SEM micrograph of silver nanoparticles synthesised by the reaction of 1 mM silver nitrate with *Sargassum cinctum* extract.

The SEM image (Fig 5) showing the high density, spherical shaped and well distributed Ag-NPs synthesized from *Sargassum cinctum* extract.

Preliminary Biochemical components, antioxidant and total phenol analysis:

The protein content of *Sargassum cinctum* is comparatively high than carbohydrates and lipid. The protein, carbohydrates and lipid content of this species was 87.17± 0.66 mg/g dry wt.; 38.49± 0.54 mg/g dry wt.; and 28.7± 0.6 mg/g dry wt. but starch content is very low, 0.1 ± 0.00 mg/g dry wt. (Fig 6)

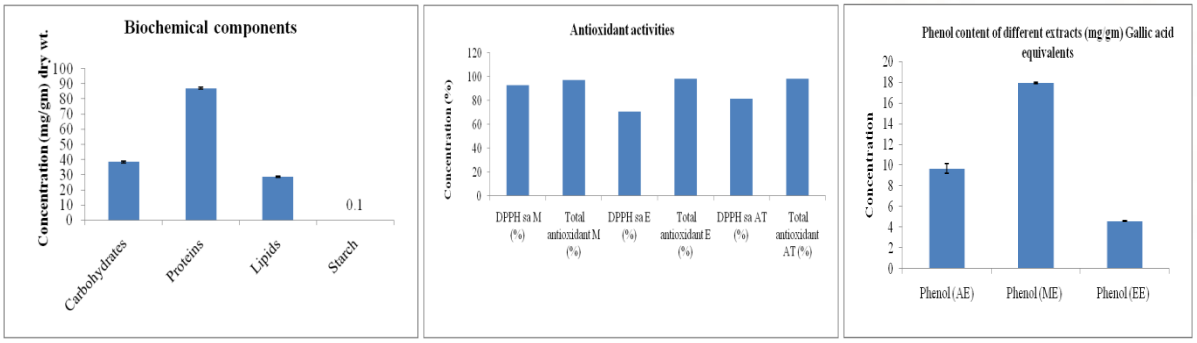

Fig 6: Graph representing the values of biochemical components;
Fig 7: Seaweed extracts anti-oxidant activity;
Fig 8: Seaweed extracts total phenol content

Three different extracts such as methanol, ethanol and acetone of seaweeds were used for the estimation of *in-vitro* anti-oxidant activities such as total antioxidant activity and free radical (DPPH) scavenging activity which revealed that methanol extract had the highest free radical (DPPH) scavenging activity (92.36%) in compare to ethanol (70.68%) and acetone (81.17%) extract, But the total antioxidant activity of three extracts were more or less equal (methanol extract-96.67%; ethanol-98.05% and acetone-98 %). It had been concluded that *Sargassum cinctum* three extracts had high antioxidant activities (Fig 7).

The total phenol content of methanol extracts (17.93 ± 0.66 mg/gm) gallic acid equivalents of *Sargassum cinctum* was the highest in compare to ethanol extract (9.65± 0.46 mg/gm) gallic acid equivalents and acetone extract (4.6 ± 0.06 mg/gm) gallic acid equivalents (Fig 8).

Seed Germination test :

The main objective of this work is to evaluate the effect of silver nanoparticles synthesized from *Sargassum cinctum* on phyto-toxicity by testing its effect on seed germination and seedling growth. After synthesis, the Ag- nanoparticles solution of seaweed was directly used for test to seed germination and the Ag-nanoparticles solution was also directly used for test to seedling growth of *Abelmoschus esculentus*. The above mentioned formula were used for the determination of germination percentage which showed that seaweed mediated Ag- nanoparticles had better germination percentage (80%) than water (40%). The seed germination parameters were measured after 24 hours, 48 hours and 96 hours. After 24 hours germination percentage was 60% and after 48 and 96 hours 80% for seaweed Ag- nanoparticles solution but in case of control it was 40 % after 24, 48 and 96 hours. Mean germination time increased with increase of time and it was maximum at 96 hours. The germination rate was the highest after 24 hours and gradually decreasing at 48 hours and 96 hours. Germination index was calculated with relative root elongation and relative seed germination and it had been observed that germination index was the highest at 48 hours (368.88) in compare to 24 hours and 96 hours.

It had been previously reported that metallic Ag-nanoparticles had dose dependent inhibitory effect on seed germination. The inhibitory effect of metallic Ag- Nanoparticles had been influenced by various factors such as size and concentration of nanoparticles, temperature, duration and methods of exposure. The dosage of 0.2 to 1.6 mg/L of Ag-NPs had been shown to inhibit seed germination, lipase activity and soluble reducing sugar content in *Brassica nigra (*Amooaghaiea et al. 2015). The 10 mg/ml Ag NPs had been reported to inhibit the seed germination in *Hordeum vulgare* and reduced shoot length in flax (*Linum usitatissimum*) and barley (*Hordeum vulgare*) (Nowack, 2010; Kaegi et al. (2010); El-Tesah et al. 2012). Some of the studies had been reported that metallic AgNPs had no significant effect on *Cucumis sativus* or *Lactuca sativa* (Barrena et al. 2009). From our study, it had been reported that seaweed mediated eco-friendly Silver nanoparticles had positive effect and it is phyto-friendly and promoting growth of seedlings and positively effected on seed germination. In compare to normal water, green synthesized silver nanoparticles had better effect on seed germination which may be due to presence of adequate amount of minerals and antioxidant and the biochemical component in seaweed extract which is used for synthesis of Ag-nanoparticles.

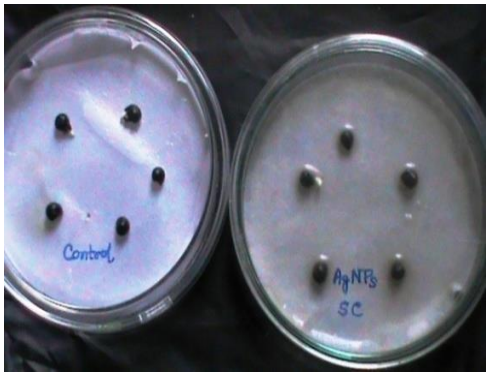

Fig 9 (a): Seed germination with normal water and seaweed Ag-nanoparticles

Fig 9 (b): Seedling with normal water as control

Fig 9 (c): Seedlings with seaweed synthesized Ag- Nanoparticles

Table 2 : Seedling height (cm) after 6 days				
Sample Id	1	2	3	Mean
Control (tape water)	12	7.5	6.7	8.73
***Sargassum cinctum* Ag-NPs**	11.2	11.5	12.3	11.66

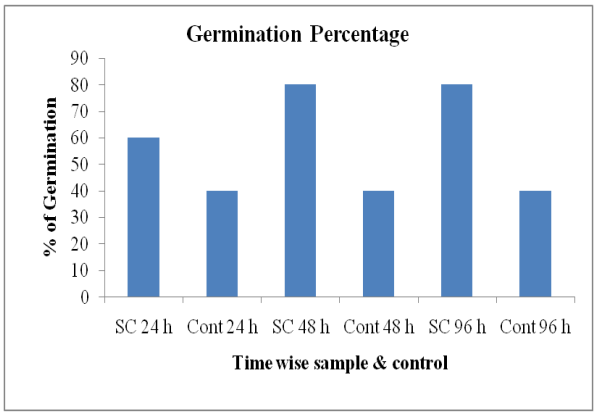

Fig 10 (a) seed germination percentage

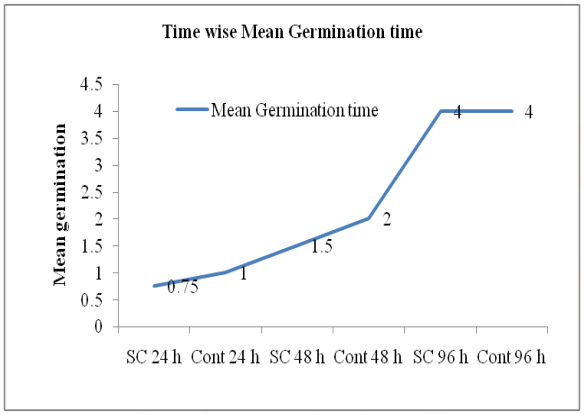

Fig 10 (b) Mean germination time

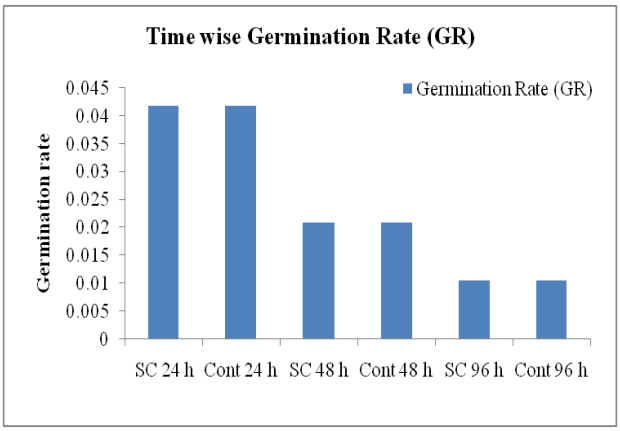

Fig 10 (c) Germination rate

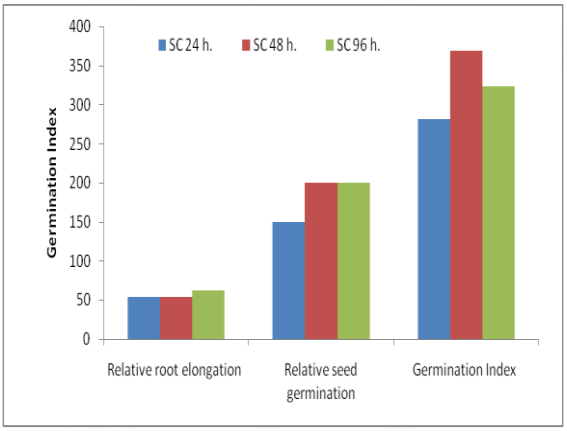

Fig 10 (d) Germination index

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Conflict of interest: There is no conflict of interest to be declared.

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