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Silver nanoparticle synthesized from *Chaetomorpha aerea* Kutz, enhancement for seed germination in *Solanum lycopersicum* L. crop

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Abstract
In this study was conducted to evaluate the effects of silver nanoparticles extracted from C*haetomorpha aerea kutz*, on seed germination application for *Solanum lycopersicum* L. crop plant. AgNPs was carried out by different instrument and technique. It includes visual observation ultraviolet–visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray analysis (EDAX), dynamic light scattering (DLS), aqueous medium containing silver nanoparticles showed absorption of the peak. Were tested Germination characteristics of Silver nanoparticles on plant growth parameters such as shoot and Root length Seed of germination and% Germination contents of economic important *S. lycopersicum* L. biological research due to their present study we have studied positive control and negative control of the germination of the seeds and impact of two concentration (0.5, 1.0, mg/L) of silver nanoparticles (AgNPs) and two concentration (100 and 500 mg/L) the concentration as compared to control (without AgNPs). We also examined the effect of silver nitrate (AgNO3), which showed that at 0.2 concentrations the plant root and shoot height was maximum observed when compared to positive and negative control and at 0.1concentration showed maximum seed germination% was observed when compared to positive and negative control in all the tested concentration.

Keywords: seaweed, silver nanoparticles, seed germination, root and shoot growth, *Solanum lycopersicum* L. crop

Introduction

Bio-nanotechnology is a new and rapidly advancing field of research lies at the interface between biology and nanotechnology (Sahayaraj and Rajesh, 2011) [1]. Silver nanoparticles transporter for deals more than carry follow as small molecule drugs to big molecule drugs to target role nanoparticles sufficient deliver to drugs for all fields they specified one of the minimum side effect (Pickup *et al* 2008) [2]. Nanoparticles are generally mentioned to as small things with one or more than surface measurements in the size range 1–100 nm. (Keller *et al* 2013; Nubia *et al* 2016) [3, 4]. Nanotechnology applications are currently being researched, tested and in some cases already applied across the entire spectrum of food technology, from agriculture to food processing, packaging and food supplements in developing countries. (Neha *et al* 2015) [5]. In our special Food Nanotechnology section we have prepared an overview of this area. Specifically in agriculture, technical innovation is of importance with regard to addressing global challenges such as population growth, climate change and the limited availability of important plant nutrients such as phosphorus and potassium. Nanotechnology applied to agricultural production could play a fundamental role for this purpose and research on agricultural applications is ongoing for largely a decade by now. This also touches on the issue of nanotechnology (Lakshmi, 2017) [6]

Biosynthesis of nanoparticle using plant extracts is the significant methods of green, eco-friendly production of nanoparticles and exploited to a vast extent since the plants with a choice of metabolites are extensively distributed, easily available and safe to handle (Nadagouda *et al* 2008) [7]. It was found to have rich total phenolics and flavonoids were quantified in the methanolic extracts. (Massoumeh *et al* 2013) [10] and evaluated the efficient of antidiabetic drug, marine algae become important source which provide several compounds of immense therapeutic potential. Alpha-amylase, alpha-glycosidase inhibitors, and antioxidant compounds (Unnikrishnan *et al* 2015) [8]. To our knowledge, there are no accessible reports on the green synthesis of silver nanoparticles from *C. aerea* kutz, therefore, the present investigation has been proposed to synthesize silver nanoparticles using water extract of green

Seaweed *Chaetomorpha aerea.* Kutz and to characterize the silver nanoparticles by UV-Visible Spectroscopy, FTIR analysis, EDAX analysis, SEM analysis, DLS analysis and XRD analysis.

To find out the seed germination percentage, shoot length and root length with seaweed extract (Positive Control), 1mM silver nitrate (Negative Control) and green Seaweed *Chaetomorph aerea Kutz. Water extract* synthesized silver nanoparticles at various concentrations on seed germination application for *S. lycopersicum L.* crop.

Material Methods

Fresh Seaweed *C. aerea kutz.* was a green algae belong to the family of *Cladophoraceae* abundantly growing in Gulf of Mannar, Southeast Coat, Rameswaram, Tamilnadu, India. The fresh seaweed was washed with running tap water in 20 minutes and dry in show at room temperature for one week. The seaweed are cut into small pieces and make fine powder, 20 g of seaweed extract are weighted and dissolved in 200ml distilled water in 500ml Erlenmeyer flask and boil for 30 mins. The extract was filtered with glass cloth again filtered with Whatman No.1, was stored in an airtight container and protected from filter sunlight for further use. 1mM of silver nitrate (AgNO3) was prepared in 1000ml bottle. The 100ml seaweed extract were mixed with 900ml silver nitrate solution (1:9) radio. The kept in dark condition and color change of the solution white with pale yellow indicated that the silver nanoparticles were synthesized. The go for centrifuge in 7000rpm and 20ºc for 15mins. Then collect the pellet and kept in hot air oven for pellet is dried. Then further use for characterization studies and seed germination studies. The characterization of silver nanoparticles was carried out by different instrument and technique. It includes visual observation ultraviolet–visible spectroscopy (UV–Vis), Fourier transform infrared (FTIR), powder X-ray diffraction (XRD), scanning electron microscope (SEM), energy dispersive X-ray analysis (EDAX), dynamic light scattering (DLS), zeta potential and antibacterial activity. UV–visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 430 nm.

Characterization of nanoparticles

UV- Vis spectroscopy analysis important for AgNPs particles characterization primary stage unique optical properties make them interact with specific wave length light absorption of the AgNPs depends upon the particles size measured in UV–visible Spectrophotometer.

FT-IR spectroscopy analysis

FTIR spectroscopy is recently used to find out biomolecules are involved in the synthesis nano materials FTIR has also been extended to the study of nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, carbon nanotubes, graphene and gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process. The silver nanoparticles were analyzed by FT-IR spectroscopy. The FT-IR spectrum was taken in the mid IR region of 400–4,000 cm-1.The spectrum was recorded using Attenuated Total Reflectance (ATR) technique. The sample was directly placed in the zinc selenide crystal and the spectrum was recorded in the transmittance mode.

XRD analysis.

The silver nanoparticles were analyzed by X-ray diffract meter (X'pert PRO PAN analyzer) with Syn Master 793 software to identify the nanoparticles product. The XRD pattern was recorded using computer controlled XRD system, JEOL, and Model: JPX-8030 with CuK radiation (Ni filtered = 13418 Ao) at the range of 40 kV, 20 A˚. The 'peak search' and 'search match' program built in software (Syn master 7935) was used to identify the peak table and ultimately for the identification of XRD peak.

Particle size analysis 10 mg of synthesized nanoparticles were taken and suspended with distilled water. Then this medium was characterized by particle size analyzer. (Prathap *et al* 2014) [9].

Seaweed Extract and its silver nanoparticle preparations for seed germination test:

Preparation of seed with *C. aerea* Kutz. green seaweed extract, silver nanoparticles and seaweed extract synthesized silver nanoparticles as bio fertilizers for seed germination. 20gms of seaweed powder was taken and was dissolved in 200ml of distilled water in a 500ml Erlenmeyear flask and boiled for 30mins. The extract was filtered with Whatman No.1 filter paper and dried the solution with the help of hot air oven. The dried substance was taken at different concentration 0.1g, 0.2g, 0.3, and 0.4g and dissolved in all the four concentration at 10ml &20ml using distilled water. The different concentration like 0.1g, 0.2g, 0.3g and 0.4g was prepared from already synthesized green seaweed silver nanoparticles in both 10ml & 20ml using distilled water.

For carrying out the further process, prepared the plate for surface sterilization in the laminar air flow chamber. Then transferred all the solutions to their corresponding plates in presence of aseptic condition and maintained all those plates in normal room temperature in the tissue culture laboratory and the observation of plants for the following 10 days were taken and the results obtained were recorded.

Seed germination measurement

The final germination percentage was calculated based on the total number of germinated seeds at the end of experiment. Based on the literature,

Total germination (Final germination percentage) (GT)

Total germination percentage (Gt) was calculated as Gt = (n/N ×100), where n = total number of germinated seeds (normal and abnormal) at the end of the experiment and N = total number of seeds used for germination test.

Germination Speed Index (GSI)

Conducted concomitantly with the germination test, with daily calculation of the number of seeds that presented protrusion of primary root with length ≥2 mm, always at the same time during the trial. The germination speed index was calculated by the sum of the number of seeds germinated each day, divided by the number of days elapsed between the seeding and germination, according to the Maguire formula (1962)

GSI = + ... + where:

GSI = seedlings' germination speed index;
G = number of seeds germinated each day;
N = number of days elapsed from the seeding until the last count.

Statistical analysis

Means and standard deviations were estimated from three replicates measurements for each treatment and the related controls. The data obtained from the various treatments was statistically analyzed.

Results and Discussion

Green Synthesis of Silver Nanoparticles

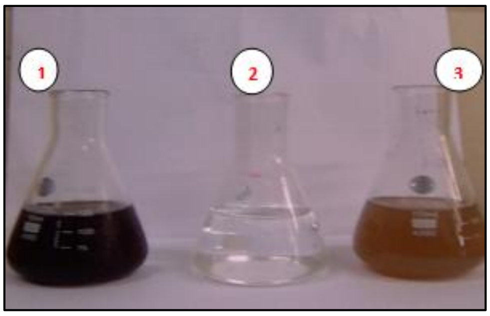

Fig 1: 1- Green seaweed water extract. **2-** $AgNO_3$ **3-** Green Seaweed synthesized silver nanoparticles solution

The brown seaweed *C. aerea kutz.* Was used for the synthesis of silver nanoparticles. Reduction of AgNO3 was visually evident from the color change of the reaction mixture. The color of the solution changed from pale yellow to brownish color. Formation of silver nanoparticles by reduction of the aqueous silver during exposure to the extract of seaweeds were *Chaetomorpha aerea* confirmed by the formation of pale yellow color after a week of synthesis (Fig. 1) which was confirmed by UV-Vis spectroscopy. The color arises as a result of the excitation of surface Plasmon vibration in the silver nano particles. This is due to the excitation of surface Plasmon resonance effect and the reduction of silver ions (Ibrahim- 2015) [11].

UV-Visible spectroscopy analysis

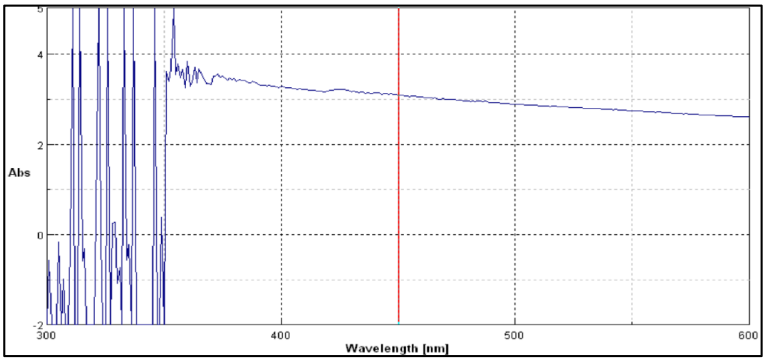

Fig 2: UV-VIS Absorption spectra of silver nanoparticles synthesized *C. aerea kutz.* Exposure with 1mm silver nitrate

The silver nanoparticles obtained were characterized by UV-Visible spectroscopy and the characteristic absorption peak at 300 to400 nm for the aqueous extract of *C. aerea kutz.* From the spectrum confirmed the formation of silver nanoparticles. Absorption spectra of silver nanoparticles formed in the reaction solution of 48 hrs of incubation has absorbance peak at 380nm, broadening of peak showed that the particles are polydispersed.

The representative spectrum of silver nanoparticles. It exhibits absorption peaks at wavelengths 3435.98(cm-1) amine group, 2076.99(cm-1) the R-N=C=S primary amines group respectively. The bond at 1634.43(cm-1) corresponding to the Amides group. The bond seen at1583.66 (cm-1) corresponding to the Aromatic ring group. The bond at 1404.32(cm-1) corresponding to the Carboxylic acid. The bond at 138455(cm-1) corresponding nitro compound group. The bond seen at 1113.79(cm-1) Ether group. The bond at 618.97(cm-1) corresponding Alkynes group (figure-2).

FTIR spectroscopy analysis

FT-IR spectroscopic studies were carried out to investigate the functional groups and Ag nanoparticles formation mechanism particularly to identify possible interaction between silver precursor salt and protein molecules, which leads the reduction of silver ions and stabilization of silver nanoparticles (Theivasanthi and Alagar 2013) [12]. FTIR spectrum comparison seaweed and silver nano particles group (Figure 3, 4, Table 1, 2) comparison. The representative spectrum of silver nanoparticles. It exhibits absorption peaks at wavelengths 3435.98(cm-1) amine group, 2076.99(cm-1) the R-N=C=S primary amines group respectively. The bond at 1634.43(cm-1) corresponding to the Amides group. The bond seen at1583.66 (cm-1) corresponding to the Aromatic ring group. The bond at 1404.32(cm-1) corresponding to the Carboxylic acid. The bond at 138455(cm-1) corresponding nitro compound group. The bond seen at 1113.79(cm-1) Ether group. The bond at 618.97(cm-1) corresponding Alkynes group, FTIR of functional groups (Table-3).

Table 1: *C. aerea kutz.* FTIR spectrum comparison

Name	Description	Name	Description
SW(Red)	*C. aerea kutz.* water extract dried pellet	NSW(Green)	*C. aerea kutz.* Water extract silver neon particle synthesized dried pellet.

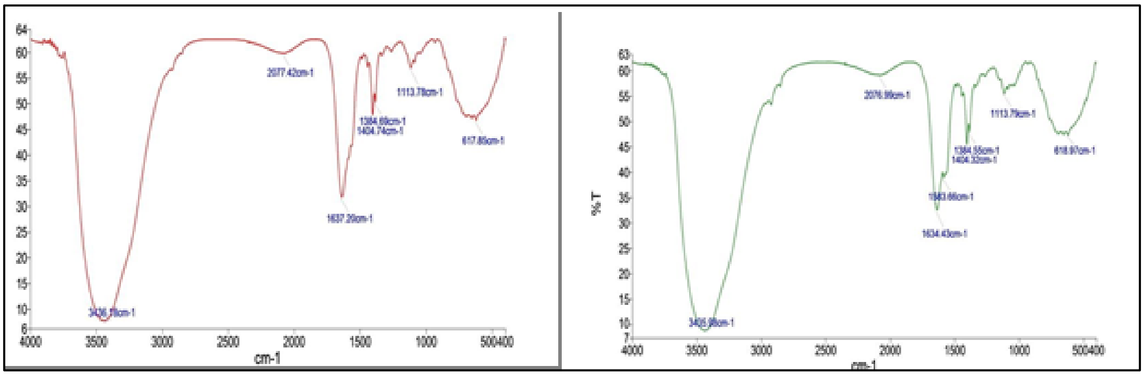

Fig 3: *C. aerea kutz.* FTIR spectrum group comparison

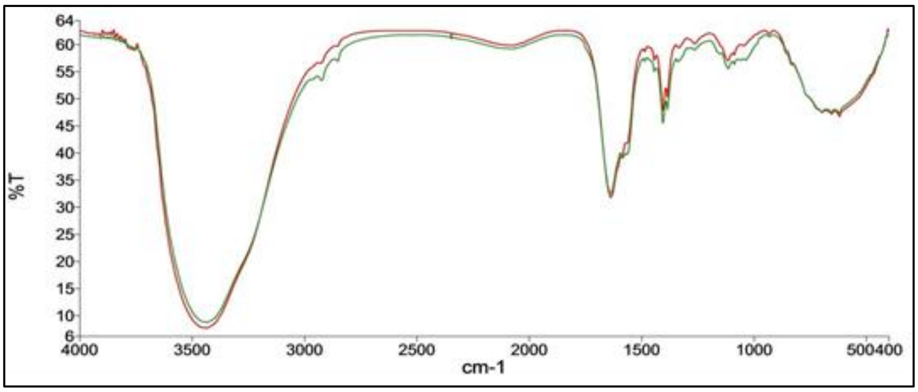

Fig 4: FTIR spectrum of silver nanoparticles synthesized from *C. aerea kutz*

Table 2: FTIR spectrum of silver nanoparticles synthesized from *C. aerea kutz.*

Name	Description
Sw(red)	*C. aerea kutz.* water extract dried pellet
Nsw(green)	*C. aerea kutz.* Water extract silver nano particle synthesized dried pellet.

Table 3: C. *aerea kutz.* dried pellet FTIR spectrum functional groups

Frequency	cm	Functional group
1	3435.98	Amides
2	2076.99	R-N=C=S_
3	1634.43	Amides
4	1583.66	Aromatic ring
5	1404.32	Carboxylic acid
6	1384.55	Nitro compound
7	1113.79	Ethers
8	618.97	Alkynes

Scanning electron microscopy (SEM)

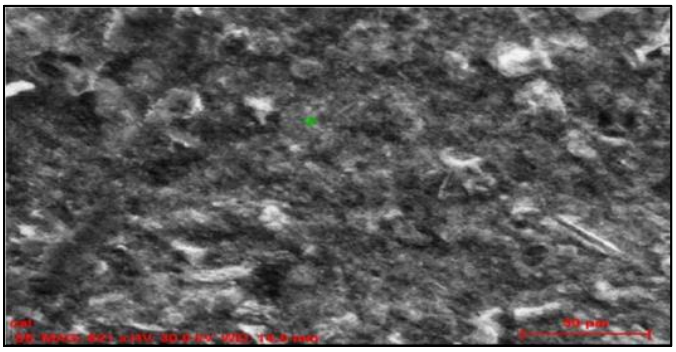

Fig 5: Scanning electron microscopy (SEM) Image showing

SEM images of the silver nanoparticles are shown in Fig. 5. The surface morphology of silver nanoparticles showed even shape and spherical nature. In the present study, the histogram of the particle size ranges width from 14 mm and MAG 621, xHV 30kv at 50 µm. The SEM image showing the high density, spherical shaped and well distributed Ag-NPs synthesized from C. *aerea kutz.* Extract strongly confirms that as a reducing and capping agent in the production of silver nanoparticles. Some results shown as a type of results Anandalakshmi *et al* 2016 [13].

Energy-dispersive X-ray spectroscopy

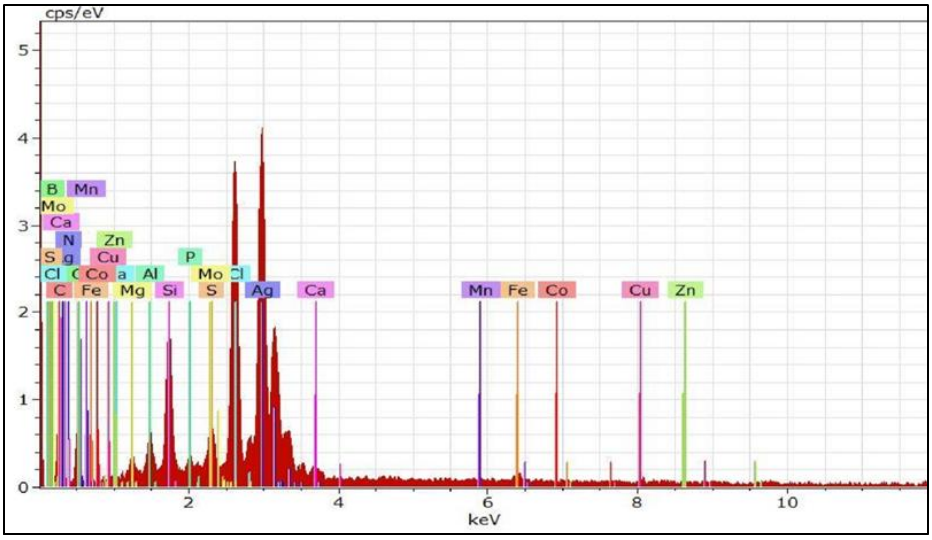

Fig 6: EDX Spectrum of silver nanoparticles synthesized from *C. aerea kutz*

The percentage of elements presents in the green seaweed synthesized silver nano particles

Table 4: El A Seriesunn. C norm. C Atom. C Error (1 Sigma)

	[wt.%]	[wt.%]	[wt.%]	[wt.%]
C 6 K series	16.78	26.39	45.70	3.80
O 8 K series	12.26	19.28	25.06	3.02
N 7 K series	3.30	5.20	7.72	1.55
Cl 17 K series	5.93	9.32	5.47	0.25
Ag 47 L series	17.74	27.91	5.38	0.62
B 5 K series	1.22	1.92	3.69	1.29
Si 14 K series	3.06	4.81	3.56	0.19
Al 13 K series	0.87	1.37	1.06	0.09
S 16 K series	0.82	1.29	0.84	0.07
Mg 12 K series	0.61	0.96	0.82	0.09
Ca 20 K series	0.29	0.46	0.24	0.05
Fe 26 K series	0.38	0.60	0.22	0.06
P 15 K series	0.15	0.23	0.15	0.04
Mn 25 K series	0.08	0.12	0.05	0.04
Cu 29 K series	0.09	0.14	0.05	0.04

Mineral analysis by EDX

C(3.80 wt.%),O (3.02 wt.%),N (1.55 wt.%),Cl (0.25 wt.%),Ag (0.62 wt.%),B (1.29 wt.%),Si (0.19 wt), Al (0.09 wt.%) S (0.07 wt%), Mg (0.09 wt.%), Ca (0.05 wt.%),Fe (0.06 wt.%), P (0.04 wt.%),Mn (0.04 wt.%) and Cu (0.05 Wt.%) of the increasing weight percentage of the minerals present in (Figure-6, Table-4) the green seaweed synthesized silver nanoparticles by the EDS analysis which is essential for seed germination and the growth of the plant.

Dynamic Light Dispersion (DLS)

Dynamic Light Dispersion (DLS) is also called photon correlation spectroscopy (PCS). The TLS uses several micron particles from the bottom of the 5 nm.

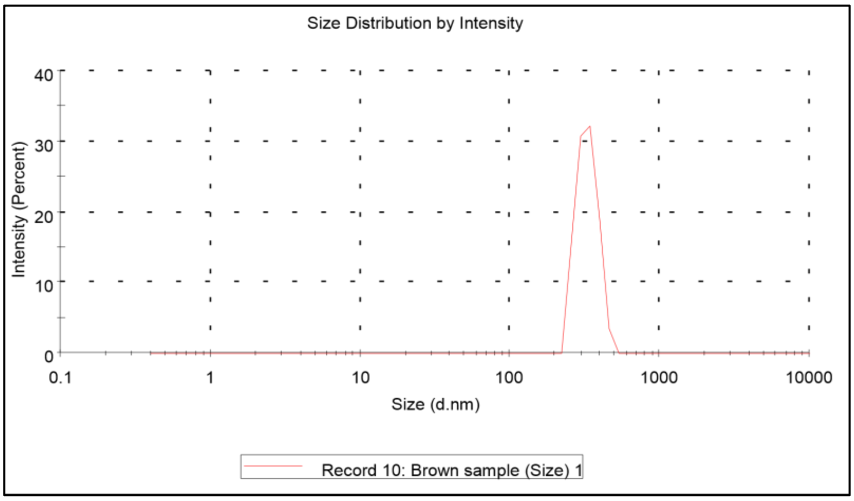

Fig 7: Particle size analyzer: DLS

Size (d.nm):% Intensity: St Dev (d.n...
Z-Average (d.nm): 394.3 v Peak 1: 328.9 100.0 51.51
PdI: 1.000 Peak 2: 0.000 0.0 0.000
Intercept: 0.718 Peak 3: 0.000 0.0 0.000
394.3nm as the (Figure-7) particle size of the green seaweed synthesized nanoparticle obtained by DLS.

X-ray Powder Diffraction (XRD)

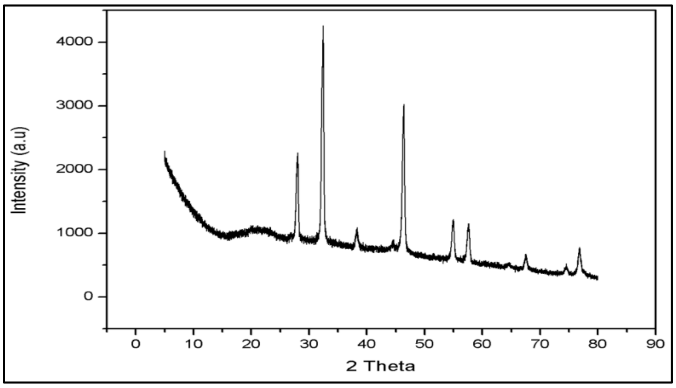

Fig 8: Pattern of green seaweed water extract synthesized silver nano particles.

The XRD analysis has shown that silver nanoparticles with well-defined dimensions could be synthesized by reduction of metal ions experiment of seed germination of nanoparticles solution with seaweed extract used as liquid bio fertilizer. Some various similarities XRD pattern of silvernano particles the peak of 38.12 degree average size of nano particles is calculated to be around the 25,o nm in that sizer then was confirmed by TEM (Harajyoti Mazumdar and G.U.Ahmed-2011) [22]. May be same biosynthesized nanostructure. The peaks observed in the XRD image at θ =28.09o, marked with (220). A number corresponding to the (220) sets of lattice planes were observed by the indexed based on crystal structure of silver nano particles. The XRD pattern thus clearly showed that the Ag-NPs are crystalline in the nature (Jegadeeswaran *et al 2012*) [21]. Both are XRD pattern that peaks are indexed as 2h positions, 10.0884 and 14.6254 plans of silver nano particles. Apart from these peaks responsible by the recorded XRD pattern shows additional unassigned peaks. They are due to the crystalline bio-organic compounds similarities observations were reported for the silver nanoparticles synthesized using *P. graveolens* leaf extract. The detailed investigation on this crystalline phase existing with the silver nano-crystals is in progress. (Prathap *et al* 2014, shiv Shankar *et al* 2004) [9, 23]

Plant root and shoot elongation of germinated seed

Fig 9: Seedling of *Solanum lycopersicum* after treatment with seaweed synthesized silver nanoparticles (SL) from *C. aerea kutz*

Table 5: AgNPs concentrations on germination percentage, germination rate and mean germination time for *Solanum lycopericum L.* crop plant

S.no	Plant length	Shoot elongation	Root elongation
P.Control-1	11.82± 2.45	2.42 ±0.51	9.4 ±2.58
N.Control-2	8.5±2	2.43±0.83	6.06±1.22
0.5 Conc.	15.83 ± 2.02	3.7 ±0.72	12.13±1.94
1.0Conc.	16.16±0.70	3.53±0.60	12.63±0.65

Table 6: Percentage of germinated seeds using seaweed extract of *Cheatomorpha aerea*

Concentrations	Germination%	
	10ml	20ml
Positive control (water)	80%	100%
Negative control (1mM silver nitrite solution)	30%	30%
0.1	30%	80%
O.2	0%	10%
0.3	0%	0%
0.4	0%	0%

Seed germination one of the dynamic phase in plant growth life cycle. Seed germination agriculture most important field. Plant roots and shoot elongation of *Solanum lycopericum* L. plant growth agent *C. aerea kutz.* Seaweed extract germinate some plant shoots and roots probably $AgN0_3$ treatment increase compare to the some plant growth regulator. Compare seaweeds $AgNo_3$ to the positive and negative control high growing different concentration of the $AgN0_3$.

Positive controlled plant mostly using water content so used measuring various concentration of the growing factor. In the positive controlled plant growth Is totally 11.82± 2.45 heights, shoot elongation is 2.42±0.51, root elongation is 9.4±2.58.

Negative control 1mM silver nitrite solution of seed germination using 1:1 concentration growing seed germination test there are compare to another content minimum growth only this part there are 8.5±2 full length plant and shoot elongation 2.43±0.83, root elongation is 6.06±1.22

Seaweeds extract AgNo3 synthesized germinated seed plants shown fully 15.83± 2.02 of heights, shoot elongation 3.7 ±0.72 these and root elongation 12.13±1.94 roots for 0.5 concentration. And 1.0concentartion of the seed germinated plants totally 16.16±0.70 heights of these 3.53±0.60 shoot and roots of 12.63±0.65. To evaluates of the roots and shoots elongation of measuring 10 to 20 day and the daily observation of crop plants. Finally crop yield under this seed germination distinctly different response was obtained for all this study during periods. (Figure-9) (Table-4) To improved agriculture area comparing other growth regulator maximum results in growth agent *C. aerea*. Kutz, bio synthesized Ag No_3 nano particles is best of this research

Statistical analysis each treatment was conducted with three replicates and the results were presented as mean ± SD (Standard deviation). The results were analyzed by one way ANOVA Mean and standard deviation

Seed germination measurement

Plant root and shoot elongation of germinated seed at 10th day

Compared to the positive and negative control 0.2 concentration of *Chaetomorpha aerea* extract silver nanoparticle synthesize showed 16.5 cm of height of germinated seed plant, of these 3.5 cm shoot height and maximum root length was 13 cm. Whereas 0.5concentration showed maximum 4.5cm shoot height (Table.1) AT Compared to the positive and negative control 0.2 concentration of *Chaetomorpha aerea* extract silver nanoparticle synthesize showed 16.5 cm of height of germinated seed plant of these 3.5 cm shoot height and maximum root length was 13 cm. Whereas 0..5 concentration showed maximum 4.5cm shoot height (Table.2).

Compared to the positive and negative control concentration of *Chaetomorpha aerea* extract silver nanoparticle synthesize showed 17 cm of height of germinated seed plant, of these 2.5 cm shoot height and maximum root length was 15cm. (Table.3).

Compared to the positive and negative control concentration of *Chaetomorpha aerea* extract silver nanoparticle synthesize showed 17 cm of height of germinated seed plant, of these 2.5 cm shoot height and maximum root length was 15cm. (Table.4).

Conclusion

Generally, metal nanoparticles are synthesized and stabilized through chemical and mechanical methods (Balantrapu *et al*., 2009*;* Tripathi *et al*., 2010) [15, 16]. Synthesis of nanoparticles through biological methods is a good, environment friendly and economically alternative method. Synthesis of green nano material's and their characterization is an emerging field of nanotechnology from the past few decades, because of their application in the fields of physics, chemistry, biology and medicine. Application of green chemistry to the synthesis of nano materials has vital importance in medicinal and technological aspects (Begum *et al*., 2009) [18]. Biologically synthesized silver nanoparticles (Ag-NPs) have wide range of applications because of their remarkable physical and chemical properties. The literature on the extra cellular biosynthesis of Ag-NPs using plants and pure compounds from plants are insignificant (Kattumuri *et al*., 2007; Gilaki 2010) [19, 20]. *Padina tetrastromatica* is abundantly growing seaweed in coastal areas of South India. It occurs in inter tidal region of Mannar, southeast coast of India. It grows in shallow sand covered rocky pools of mid-and lower littoral zones (Jegadeeswaran *et al* 2012) [21].

In the present study silver nanoparticles were synthesized from aqueous seaweed extract of *Chaetomorpha aerea.* Primarily synthesis of silver nanoparticles was confirmed by pale yellow colour formation which indicated the reduction of silver ions by seaweed extract. Characterization by the UV-Vis, FTIR, XRD, SEM and EDAX instrument analysis confirmed the presence, size, stability and minerals presence in the synthesized silver nanoparticles. After characterization, the silver nanoparticles were tested at various concentration to check the seed germination, root, shoot height and showed best results and in further it can be used as bio fertilizer as a greener approach.

In this work, *Lycopersicum esculentum* bioassays employed in our experiments provided valuable information concerning the application of AgNPs on vegetable plants in the environment and as of AgNPs, possible uptake and translocation of nanoparticles observed in this study.

At 0.2 concentrations the plant root and shoot height was maximum observed when compared to positive and negative control and at 0.1concentration showed maximum seed germination% was observed when compared to positive and negative control.

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