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Green synthesis and characterisation of silver nanoparticles using aqueous extract of *Gracilaria corticata* and their effect on growth of beneficial soil microbes and earthworms

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ABSTRACT

Nanoparticles (NPs) of $AgNO_3$ are receiving increasing attention due to their widespread applications. In this report, the green synthesis of silver nanoparticles by using marine red seaweed *Gracilaria corticata* was discussed. The synthesized nanoparticles scrutinized by UV-Visible spectrophotometer shows the crest at 420nm. X-ray crystallography confirmed the silver nanoparticles crystalline nature. The structure and size of the silver nanoparticles were examined by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) in the average amount size is 25nm clearly shown and most of the nanoparticles were cubical and hexagonal in shape. The compounds responsible for the silver nanoparticles biosynthesis were studied using Fourier Transmission –Infrared Spectroscopy (FT-IR). With increasing number of applications of NPs, assessing the risks posed by Nps has become a research area that more scientists are interested. In this study, effect of AgNps biosynthesized by aqueous extract of *Gracilaria corticata* on soil microbes and the earth worm (*Lumbricus rubellus*). The size of Grc-AgNps range from 1-25nm, at this size, the particles' surface area is large to its volume, which enables its increased reactivity and toxicity against soil microbes (*Bacillus* spp*, Bacillus substilis, Staphyllococcus epidermidis, Serratia* spp**,** *Pseudomonas fluorescens***,** *Pseudomonas* spp*, Aspergillus flavus***,** *Aspergillus fumigates***,** *Alternaria altermata***,** *Cladosporium* spp). The exposure of Grc-AgNps treatment reduced the pigmentation of the skin and growth of the earthworm.

Introduction

Silver nanoparticles (AgNPs) have been shown to have powerful bactericidal properties even in mild concentration. *In situ* studies have demonstrated that silver, even in larger particle form, inhibits microbial growth below concentrations of other heavy metals. Toxicity of nanosilver has been reported in heterotrophic (ammonifying/nitrogen fixing/PGPR) and chemolithotrophic, soil formation bacteria (Throback *et al*., 2007). However, the actual mechanism by which AgNPs inhibit bacterial growth is still not unclear. Moreover, Soni and Bondi (2004) reported that AgNPs damaged and pitted the cell wall of *E. coli* and accumulated in the cell wall, leading to increased cell permeability and ultimately cell death (Soni and Bondi, 2004). On the other hand reports suggest bactericidal effect of nanosilver by destroying the enzymes that transport the cell nutrient and weakening the cell membrane or cell wall, leading to increased cell permeability and cell death (Zeng *et al.,* 2006). However, other researchers believe nanosilver destroys the ability of the bacterial DNA to replicate. Size of nanosilver range from 1-50nm, at this size, the particles' surface area is large comparative to its volume, which enables its increased reactivity and toxicity against bacteria and various microbes. In addition, nanosilver of 1-10nm range exclusively attaching to the HIV-I virus and inhibiting it from binding to hosts cells (Doshi *et al.,* 2008).

The potential for nanosilver to adversely affect beneficial bacteria in the environment, especially in soil and water, is of particular concern. In recent years concerns have been mounting that AgNPs pose an unacceptable toxicity risk to

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human health and the environment. Conversely, there is also a risk that use of AgNPs will lead to the development of antibiotic resistance among harmful bacteria. As a powerful bactericide, AgNPs threaten bacteria dependent processes that underpin ecosystem function. Beneficial bacteria are of vital importance to soil, plant and animal health. In order to detect the ecotoxicity of environmental chemicals, many studies have used bacteria and earthworms (Gooneratne *et al*., 2011; Pasco *et al*., 2011). The biosynthesis of silver nanoparticles via a single–step reduction of silver ions using renewable and biodegradable seaweed extracts at room temperature without the use of any reducing or capping agentsis reported here. Characterization of the synthesised nanoparticles utilizing UV-visible spectroscopy, Scanning electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy analysis and Transmission electron microscopy besides their effect on beneficial soil microbial isolates and the growth of earthworms are presented.

Materials and Methods

Collection and preparation of seaweeds for analysis

The red marine algae *Gracilaria corticata (*J. Agardh) J. Agardh (Rhodophyceae) was collected from Punnakayal, Thoothukudi District, Tamil Nadu. Seaweed was collected during low tide in the forenoon during January 2016. The collected seaweed was identified on the basis of pigmentation, morphology and authenticated by Dr.P.Anantharaman, Associate Professor CAS in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu. The collected material was washed with seawater and immediately transported to a laboratory in polythene bags containing natural sea water to prevent evaporation. Algal material was washed with distilled water to remove the dust and soil. After cleaning, the fresh algae were shade dried at room temperature for a week. Dried seaweeds was powdered in the mixer grinder.

Preparation of seaweed extract

The seaweed powder (5g) was soaked for 24h in 1L of sterile water. Then the crude extract was blended thoroughly and filtered using a Whatman No.1 filter (24ìm) twice. The filtrate was used for further analysis.

Synthesis of silver nanoparticles

In the seaweed extract, 1mM silver nitrate solution was added. The reduction of silver nitrate occurred within 10min which resulted in colour change (dark brown), as noted by visual observations indicating the formation of AgNPs. As per the absorption spectrum, this medium remained stable for more than 3 months. The absorbance of aliquots of the reaction solution was measured using a UV-2371 spectrophotometer operated at a resolution of 1nm (Kumar *et al.,* 2012).

Antioxidant activity

Seaweeds contain many phytochemicals including compounds with antioxidant activity, which are mostly phenolic compound (Walailuck *et al.,* 2011; Aliyu *et al.,* 2009). Compounds with antioxidant activity are mainly phenolic acids, flavonoids and polyphenols, so content of total phenol **(**Duan and Binachi, 2006), flavonoid **(**Zhinshen *et al.,* 1999), tannin (Julkunen-Titto, 1985) terpenoid (Ramani *et al.,* 2011; Abdul Wadood *et al.,* 2013) and tocopherol (Rosenberg, 1992) were investigated in *Gracilaria corticata* and *Grc*-AgNPs (aqueous extract).

The different techniques were used to characterized the Grc-AgNPs such as UV-vis (2371) used to know the band at nanometer, X-ray diffractometer (JEOL) used to determine the crystallinity structures of silver nanoparticles, FT-IR (Fourier Transform Infrared spectroscopy) from (Systronics 166) type FTIR spectrometer which was used in the range 400 to 4000 cm^{-1} by KBr pellet method for *Gracilaria corticata* extract powder and silver nanoparticles, TEM (JEM 2100) and SEM ((VIGA 3-30.0 HV SEM) analysis were carried out to confirm the image of specimen by magnified focus on imaging device.

Isolation of soil microbes (Robert *et al.,* 1957; Berg and Ballin 1996)

The isolation of microorganisms was carried out using a serial dilution technique. Aliquots of 100µL of different dilutions of garden soil were spread onto plates of nutrient agar medium for bacteria and potato dextrose agar for fungi. The plates were incubated at 28°C for 5 days under aerobic conditions. Developed colonies were picked and isolated following morphological criteria. Purified isolates were obtained by repeatedly streaking colonies on a TSA (Trypticase soy agar medium) and observing them using light microscopy. The identification and classification of the colony morphotypes were achieved using five parameters: colony size, form, colour, texture and margin. The isolated bacterial and fungal colonies were identified by Dr. D. Arvind Prasanth, Assistant Professor of Microbiology, Periyar University, Salem, Tamil Nadu. The effect of synthesized nanoparticles on soil microbes were tested using these isolated microbial colonies.

Antibacterial assays

Colorimetric broth assay (Mishra and Kumar, 2009)

Overnight cultures of microbial isolates were subcultured in the nutrient broth. Samples of 3ml of microbial culture were placed into test tubes and 1, 1.5 and 2ìl of appropriate dilutions of *Grc*-AgNPs were added. After 24 h incubation at 37 °C, the optical density (OD_{520}) was measured using the spectrophotometer. The MIC (Minimum Inhibition Concentration) for growth was defined as the lowest concentration of NPs, which inhibited bacterial and fungal growth.

Agar well diffusion assay for bacteria (Robert *et al.,* 1957).

The antibacterial assays were done on the garden soil bacterial isolates by standard well method. Nutrient broth/agar medium was used to cultivate bacteria. Fresh overnight cultures of inoculums of each culture were spread on to nutrient agar plates using sterile cotton swabs. The well was made in the agar plate using cork borer. The silver nanoparticles along with the sample (2 µg) were poured over the well of inoculated plates followed by incubation overnight at 37ºC. The antibacterial activity was assigned by measuring the diameter of the zone of inhibition around the well.

Agar well diffusion assay for fungi (Berg and Ballin 1996)

Antifungal activity *Grc*-AgNPs against garden soil fungal isolates were determined by using well agar diffusion method. Stock cultures were prepared and maintained in Potato dextrose agars were also done parallel. The plates were examined for evidence of zone inhibition, which appear as a clear around the well. The diameter of such zone of inhibition was measured using a meter ruler. Mean value was calculated by performing the experiments in triplicates.

SWC preparation to study the growth of earthworm and soil properties (Senevirathne *et al.,* 2006)

10 g of powdered *Gracilaria corticata* seaweed were added with distilled water in the ratio of 1:10 and was autoclaved at 15 lb pressure for 1hour. Extract obtained from the seaweeds were filtered immediately through a muslin cloth. The extract was weighed, labelled and stored in a refrigerator. The extract thus obtained was considered as 100% seaweed concentrate (SWC). SWC of 1% was prepared with distilled water and used for the study.

Effect of silver nanoparticle on the morphology and histology of earthworm (Perez-Losada *et al.,* 2009)

The earth worms (*Lumbricus rubellus*) were taken from 5 cm depth of the field from Aniyaparanallur, Srivaikundam and Thoothukudi. Earthworm of adult weighing about 200-300mg and having well developed clitellum were used for all the experiments and they were grown in earthern pots containing native soil to maintain physical and chemical parameters. 500 g of soil was added to each of the pot. The pot is in 37cm diameter and 17cm length. Without any application was maintained as control. The reference control also kept with the application of SWC (*Gracilaria corticata*). Another set kept with the application of *Grc*-AgNPs was considered as treatment. The soil samples were drawn in the earthern pots to determine the physical (soil moisture and bulk density) and chemical (soil pH, organic matter) characteristics on 20th and 40th days after treatment. The coconut fibre was also placed to prevent the earth warms from crawling out. Ten adult earthworms were added uniformly in control and treatment pots. Few earthworms were randomly observed at 20th and 40th days for the study of morphology and histology of earthworm.

Transverse sections of segments from the clitellum region of earthworms (*Lumbricus rubellus*) exposed to green synthesize AgNPs

The histology of clitellum of earthworm was studied adopting the routine paraffin method (Humason, 1979).

Statistical analysis

Results obtained in this study have been subjected to Standard deviations using computer software.

Result and Discussion

Synthesis and characterization of silver nanoparticles

The UV-vis spectra of AgNPs synthesised by *G.corticata* are shown in Plate-1. UV-visible spectrum of reaction mixture at different wavelength ranging from 416-591nm Fig.1 showes strong absorbance peak at 375-490 nm indicates the formation of AgNPs Fig.1 confirms the formation of silver nanoparticle (Baia and Simon, 2007; Ayman *et al.,* 2014). The peak indicated a surface plasmon resonance (SPR), which has already been recorded for various metal

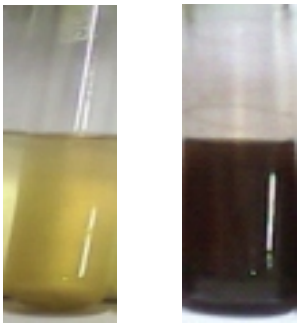


Plate-1. *Digital photograph showing in the colour change of AgNO3 on addition of aqueous extract of seaweeds*

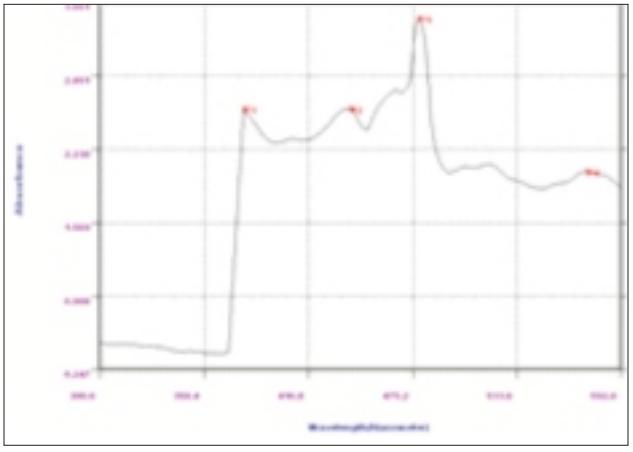


Fig. 1. *UV-Visible spectra of the synthesised silver nanoparticles from the aqueous extracts of seaweeds* Grc-*AgnP*

nanoparticles which ranged from 2 to 100nm in size (Henglein, 1993; Ravindra and Rajasab, 2014).

Antioxidant activity

The antioxidant capacity observed on the total phenols, tannins, flavonoids, tocopherol and terpenoids content. The substantial amount of antioxidants in aqueous extracts of *Gracilaria corticata* and *Grc*-AgNPs presented in (Fig.2), indicated these chemical content were predominantly higher in aqueous extract of *Gracilaria corticata* compared to the seaweed reduced *Grc*-AgNPs, But terpenoid content higher in *Grc*-AgNPs were noted Polyphenolic compounds are natural antioxidants which are found mostly in seaweeds (Moon and Shibamoto,2009).

FT-IR analysis of silver nanoparticles

The presences of some functional groups are revealed by FTIR spectral analysis is shown in Fig. 3. FTIR spectrum of *G. corticata* before and after reaction with silver nitrate were represented in Fig.3(a). Control shows different major peaks positioned at 1466.81 3446.71, 1637.21cm^{1} were assigned to stretching vibration of N-H of amine, -C-C- of alkanes and C=C of alkenes, respectively (Socrates, 1980). After reaction with $AgNO_3$ there was a shift in the following peaks 1359, 1614.43, 2853.65, 3277.03cm^{1} indicating that C-H of alkanes, -C-C- of alkanes, O-H alcohol phenol stretch, and blending of aromatic ring on the surface of *G.corticata* may be participating in the process of nanoparticle synthesis (Kannan *et al.,* 2013). (Fig.3 (b).

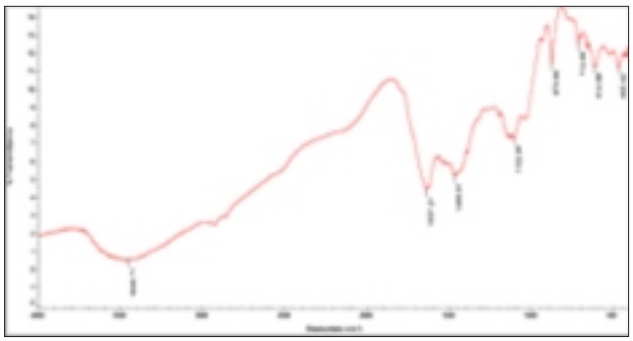


Fig. 3(a). *FT-IR spectra of* G.corticata

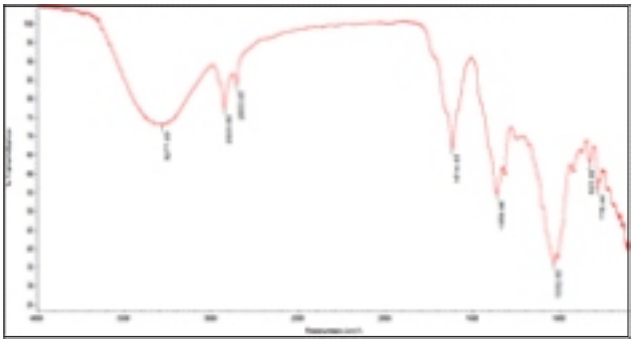


Fig. 3(b). *FT-IR spectra of* Grc-*AgNPs*

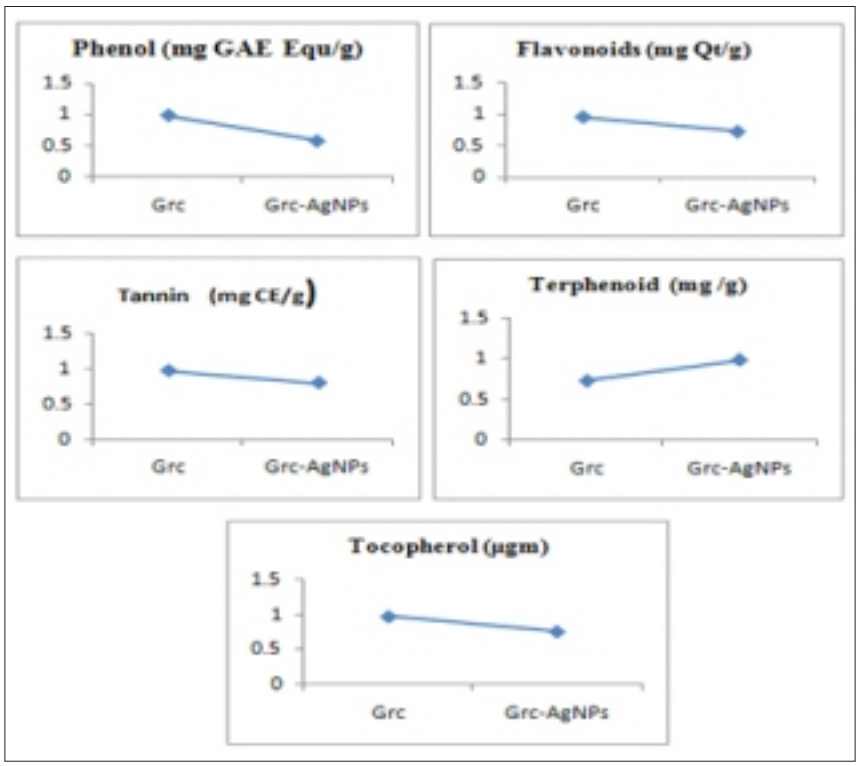


Fig. 2. *Comparison of amount of antioxidant contents in seaweed and seaweed synthesis silver nanoparticles*

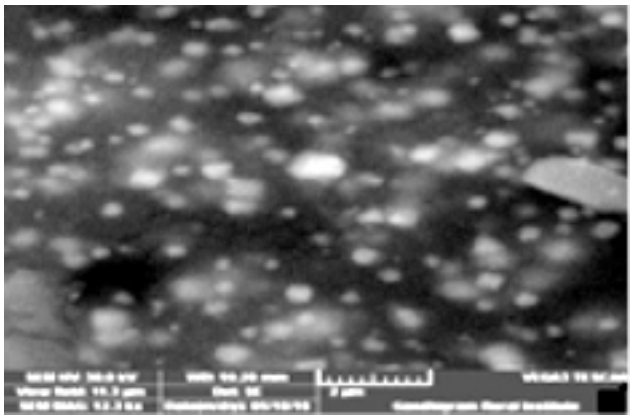


Plate-2. *Scanning Electron Microscopy (SEM) micrographs of synthesised G.corticata silver nanoparticles (Grc-AgNPs).*

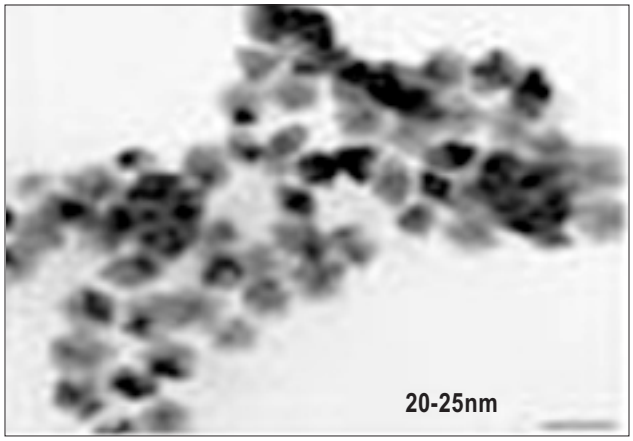


Plate-3. *Transmission Electron Microscopy (TEM) micrographs of synthesised seaweeds silver nanoparticles* Grc-*AgNPs*

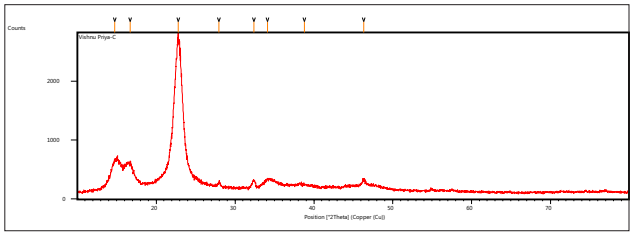


Fig. 4. *XRD diffraction patterns recorded from drop coated films of silver nanoparticles on* Grc-*AgNPs.*

SEM analysis

The SEM monographs of *Grc*-AgNPs in the filterate shown in (Plate-2). The cubical and hexagonal shaped AgNPs observed and it was found to be 25-50nm range in size (Singh *et al.,* 2010).

TEM analysis

The TEM image shows the shape and size distribution (Plate-3), the particles were comparatively hexagonal and cubical in shape with a diameter of 20-25nm.

XRD analysis

The XRD pattern of the peaks (Fig. 4) shows that the particles are crystalline with small size. The lattice planes (111), (200), (220) and (311) were identified with the corresponding Bragg reflections with 2è values of 38, 44, 6 and 77 respectively, which confirm the face-centered cubic structure of the formed AgNPs (Thevasanthi and Alagar, 2010; Zargar *et al.,* 2011).

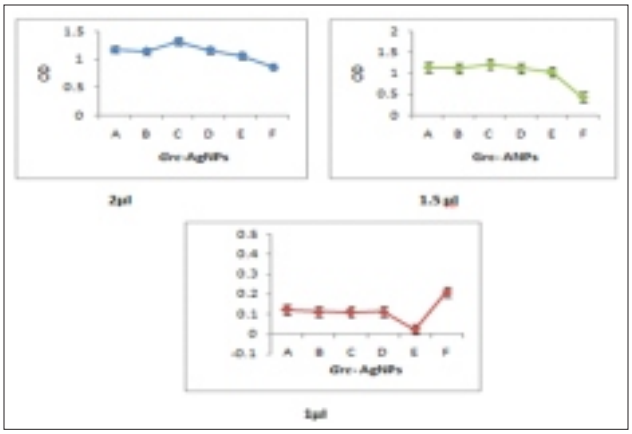
Effect of green synthesised nanoparticles *Grc*-AgNPs on soil microbial isolates

The effect of *Grc*-AgNPs on the microbial isolated from garden soil were studied. The isolation of microorganism was carried out using serial dilution technique. Aliquots of 100 µl of different dilution of garden soil were spread onto plates of nutrient agar medium for bacteria and potato dextrose agar for fungi. The plates were incubated at 28º C for 5 days under aerobic conditions. Developed colonies were picked and isolated based on morphological criteria and the isolated bacteria were sub-cultured as pure culture. Pure cultured microbes were identified as *Bacillus spp, Bcillus subtilis, Staphylococcus epidermidis, Serratia spp, Pseudomonas spp, Pseudomonas fluorescens* and isolated fungi were *Aspergillus fumigates, Aspergillus flavus, Alternaria alternata, Cladosporium spp* **.** The bacteria isolated from the garden soils are soil N-cycle, nitrifying bacteria (Mishra and Kumar, 2009). To study the effect of silver nanoparticles on soil microbes 2 type of *in vitro* assay were carried out they are calorimetric broth assay and agar well diffusion assay.

Calorimetric broth assay

Ten different microbes isolated from soil such as *Bacillus spp, Bcillus substilis, Serratia spp, Pseudomonas fluorescens, Pseudomonas spp, Staphylococcus epidermidis, Aspergillus fumicatus*, *Aspergillus flavus*, *Alternaria alternata*, and *Cladosporium spp* using calorimetric broth and microbial assay and agar well diffusion technique.

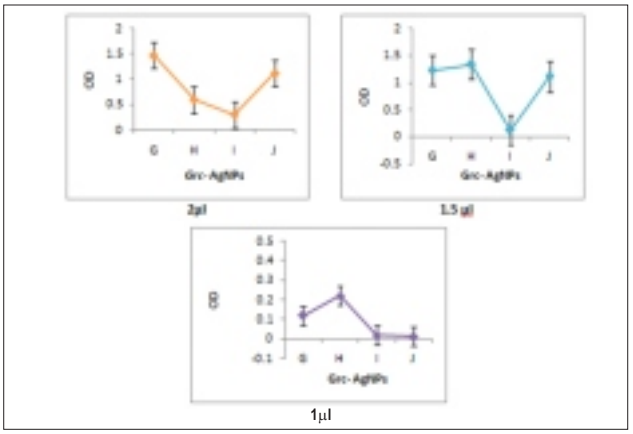
Overnight cultures of these microbes were subcultured in a nutrient broth. Sample of 3ml of microbial culture were placed into test tubes and 1, 1.5 and 2µl of appropriate dilutions of *Grc*-AgNPs were added. After 24 hours incubation, absorbances reading at 520nm wavelength for seaweed were measured post incubation at 37º C for 12 hours. Baterial cell viability and minimum inhibitory concentration (MIC) values were determined by observing the turbidity and the absorbance reading of the suspension post incubation. The lowest concentration of synthesized nanoparticles with clear suspensions was considered as the MIC values. The suspensions of isolated microbial inoculums with all different concentrations (1µl, 1.5µl, 2µl) of *Grc*-AgNPs in broth assay method were very cloudy (Fig. 5-6) and that remained throughout the incubation period. This observed the visual suspension for determining the MIC as the turbidity due to



Values are mean of 3 replicate ± SD

A- *Bacillus spp;* B- *Bacillus subtilis;* C- *Staphyllococcus epidermidis;* D-*Serratia spp;* E- *Pseudomonas fluorescens;* F- *Pseudomonas spp*

Fig. 5. *Bactericidal activities of 2µml, 1.5µml and 1µml of .silver nanoparticles on bacterial isolates*



Values are mean of 3 replicate ± SD

G-*Aspergillus flavus;* H- *Aspergillus fumigatus;* I- *Alternaria altermata;* J- *Cladosporium spp*

Fig. 6. *Fungicidal activities of 2µml, 1.5µml and 1µml of silver nanoparticles on fungal isolates.*

bacterial and fungal growth. The calorimetric broth microbial growth assay gave MIC value is 1 µl (Fig. 5) for all the tested 6 bacteria and 4 fungi. 1µml of *Grc* -AgNPs showed maximum growth inhibitory activity on *Pseudomonas fluorescens.* The results of calorimetric broth microbial growth assay (Fig. 6) showed that more inhibition (less turbid mixture) on *Cladosporium spp*. The results of the present study showed significant antimicrobial activities in calorimetric broth assay.

The result of the agar well diffusion assay tests of *Grc*-AgNPs were tested against soil microbial isolates using agar well diffusion technique. The synthesized AgNPs were found to be ineffective or showed poor inhibition on *Bacillus spp, Bcillus substilis, Pseudomonas fluorescens, Pseudomonas spp, Staphylococcus epidermidis, Aspergillus flavus*, *Alternaria alternata*, and *Cladosporium spp* bacterial and fungal growth. The larger zone of inhibition was observed on *Serratia spp* (7mm) and *Aspergillus fumigates* (5mm). Though minimum inhibition on soil microbes by synthesized silver nano particles have been observed in our study. Findings suggest that bacteria with a tolerance for a toxic agent may appear with time (Baath,1992) and the antimicrobial activity of synthesised NPs could possibly be reduced by bacterial self protection mechanism for instance. The idea that microorganisms are resistant, resilent, and functionally redundant is pervasion in ecology (Allison and Martiny, 2008). The AgNP-toxicity to nitrification bacteria has been reported to be highly dependent on their size, where AgNPs with less than 5nm diameter were reported to significantly inhibit the nitrification bacteria (Cha and Hu, 2008). The present result showed that the average particle size is 25 to 50nm. AgNPs interactions with bacteria have been found to be dependent on the size and shapes of the NPs. AgNPs have spherical (7and29nm) and Pseudospherical shape (89nm) with a narrow size distribution. Among these, Martinez-Castannon *et al.* (2008) found that the 7nm AgNPs presented best activity against *E.coli* and *S.aureus*. Because of their size, 7nm AgNPs can easily reach the nuclear contact of bacteria and they present the greatest surface area. Therefore the contact with bacteria is the greatest (Lok *et al.*, 2006). Basically, the smaller size they are, the greater their surface area to volume ratio and higher their microbial contacting efficiency (Wong *et al.,* 2010).

Effect of silver nanoparticles on soil parameters and Earthworm (Morphology and Histoloy)

Lumbricus rubellus is the most common worm species found in agricultural ecosystems (Perez-Losada *et al.,* 2009). Therefore this species is an interesting candidate for use as an animal model to monitor soil pollution. To study the effect of seaweed synthesis AgNPs on soil, experiments were carried out on *Lumbricus rubellus* earthworms. Soil samples were drawn before and after *Gracilaria corticata* and *Grc*-AgNPs application to access physical (moisture content and bulk density) and chemical parameters (pH and organic matter) of the soil. A trend of decrease of bulk density was observed with the addition of SWC (Table-1) and *Grc*-AgNPs were recorded and were increased with days of treatments.

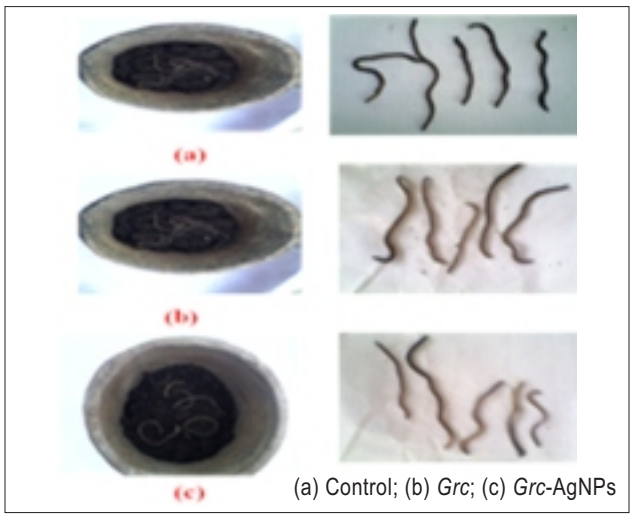
The soil organic matter increased gradually in the control and reference control as days proceeded (Table-1). But application of SWC increased the organic matter in all the soil samples and the increment was rapid with increasing growth period. Decreased moisture content, Bulk density and organic matter induced by silver nanoparticles reduced by seaweeds in the present study is consistent with earlier reports (Cornelis *et al.,* 2012; Benoit *et al.,* 2013) and (Wang *et al.,* 2013). Soil with

Table-1. *Effect of application of silver nanoparticles synthesised by seaweeds on soil properties*

No.	Treatment	pH			Moisture content (%)			Bulk density (g/cm3)			Organic matter (%)		
		0th day	20th day	40th day	0th day	20th day	40th day	0th day	20th day	40th day	0th day	20th day	40th day
1.	Control	6.92±0.86	7.07±0.76	7.40±0.003	31±0.023	34±0.034	34± 0.19	1.40±0.9	1.40±0.29	1.40± 0.76	4.7±0.6	6.0± 0.26	7.40±0.17
2.	*Gracilaria corticata*		7.35± 0.36	7.64±0.231		34±0.247	34± 0.26		1.39± 0.76	1.31±0.56		7.52±0.46	8.22±0.10
3.	*Grc*-AgNPs		7.23±0.006	7.72±0.054		34±0.017	16±0.012		1.30± 0.32	1.20± 0.02		5.55±0.2	6.40±0.1

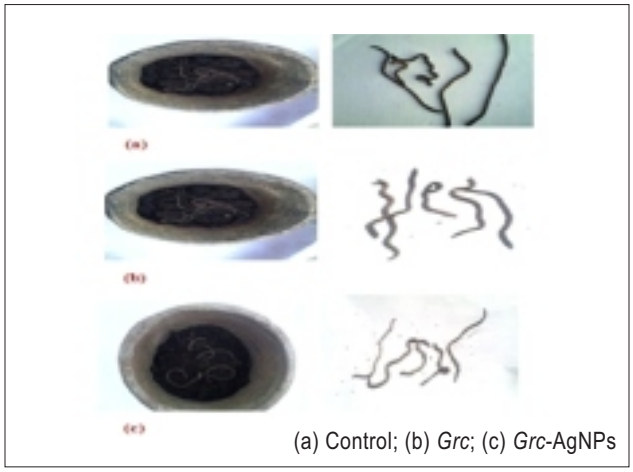
fine texture was reported to exhibit higher surface areas which also facilitate Ag-sorption (Jacobson *et al.,* 2005). Ag sorption and mobility are also controlled by soil organic matter(Jones and Peterson, 1986). Soils with high organic matter concentrations sorb Ag more strongly than to mineral soils. *Lumbricus rubellus* earthworm genus, exposed to Ag-NPs, and morphology and histology were observed on every 20th and 40th days. One set of reference control were kept with application of SWC.

The exposure of *Grc*-AgNPs had no effect on earthworm survival over the 40 day period. There was 100% survival of the nanoparticles exposures. The present results are in agreement with the findings of Roh *et al.* (2009). But after 20 days of exposure of AgNPs the brown pigmentation of earthworm's skin gradually changed into gray colour. The pigmentation is gradually decreased with increased period in NPs treated earthworm (Plate 4-5).



(a) Control; (b) *Grc*; (c) *Grc*-AgNPs

Plate-4. *Effect of* Grc *and* Grc-*AgNPs on the growth of earthworm in earthern pot (Photo taken on 20th Day)*



(a) Control; (b) *Grc*; (c) *Grc*-AgNPs

Plate-5. *Effect of* Grc *and* Grc-*AgNPs on the growth of earthworm in earthern pot (Photo taken on 40th Day)*

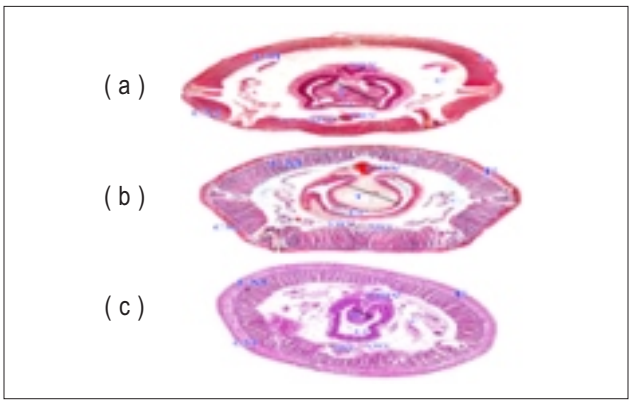


Fig. 7. Transverse sections of segments from the clitellum region of earthworms (*Lumbricus rubellus*) exposed to green synthesize AgNPs

(a)Control; (b) *Grc;* (c) *Grc*–AgNPs; LI-Longitudinal muscle; E-Epidermis;

(b) C – Coelom ; CM- Circular muscle ; SBV – Subneural blood vessel ; VBV - Ventral blood vessel ; LI – Lumen of intestine ; T – Typhlosole ; DBV – Dorsal blood vessel

The histology studies on earthworm gave the conclusion that the green synthesised nanoparticles showed high damage in the intestinal wall (Fig. 7). No other abnormalities were observed in control and reference control category of earthworms. The AgNPs found to be having negative effect on the earthworm.

Over the past decades, the usage of silver containing medications has become more prevailing because of their potential antimicrobial activity and people are choosing silver containing medicines as an alternative health supplement. Further, they are widely used in a range of materials and consumer products, including plastics, textiles, surface coatings on buildings and cosmetics. Their use is increasing and they are therefore more likely to be released to the environment where they may have damaging effects on ecologically-important bacteria and other living organism. So, some guidance is needed as to which precautionary measures are warranted in order to encourage the development of " green nanotechnologies" and their further innovative technologies, while at the same time minimizing the potential for adverse effects on human health and/or the environment. Thus, there is urgent need for a systematic evaluation of the potential adverse effect of nanotechnology. It is therefore recommended that the ecotoxicological effect of nanoparticle be clarified before their application.

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