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# INVITRO GERMINATION EFFECT OF BIOLOGICALLY SYNTHESIZED SILVER NANOPARTICLES FROM SARGASUM PLAGIOPHYLUM ON ARACHIS HYPOGAEA,VIGNA MUNGO, VIGNA RADIATE

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## ABSTRACT

*Nanobiotechnology deals with the work at nano sized level. The nanoparticles are synthesised from various sources, Eventhough use of biological synthesized nanoparticles are fascinating now a days. The present work deals with the synthesis and characterization of silver nanoparticles by biological means from marine algae Sargasum plagiophylum. The synthesized material were prepared at different ppm10, 50,100,150,200 and used as Invitro growth promoting factor for the growth of Arachis hypogaea,Vigna mungo, Vigna radiate. The shoot, root length and the protein profiles were determined.*

**KEYWORDS –** Biosynthesis, Characterization, Growth promoter, Protein profile, *Sargasum plagiophylum,*

## INTRODUCTION

Nanoparticles are synthesized by physical, chemical and biological methods. Green Biosynthesis of nanoparticles from green biological source is an important area in the field of nanotechnology which has economic and eco-friendly than other method of synthesis. Toxicity problem can be overcome by follow the green synthesis method. Several methods have been used for the green synthesis of NPs using microorganisms, marine micro and macro organisms, algal and plant extracts.

Nanoparticles are classified primarily into two types, viz organic and inorganic nanoparticles.The nanoparticles of carbon are called the organic nanoparticles. Magnetic nanoparticles, noble metal nanoparticles (platinum, gold and silver) and semiconductor nanoparticles (titaniumdioxide, zinc oxide and zinc sulfide) are classified as inorganic nanoparticles [1].

The phenolic content of brown algae varies from 20-30% dryweight [2]. Antioxidant compounds play an important role against various diseases such as atherosclerosis, chronic inflammation, cardiovascular disorders, cancer and aging processes [3]. Among brown seaweeds, *Sargassum* had been studied extensively for its high antioxidant potential *in vitro* [4].

From the time immemorial the macroscopic marine algae have been closely associated with human life and are being exhaustively used in numerous ways as a source of food, feed, fertilizer, medicine and chiefly for economically important phycocolloids[5]. Nanoscience has taken scientists around the world by storm. It claims to revolutionize the world we live in with radical breakthroughs in areas such as materials and manufacturing, electronics, medicine and healthcare, environment and energy, chemical and pharmaceutical, biotechnology and agriculture, computation and information technology [6].

Nanoparticles received a particular attention for their positive impact in improving many sectors of economy, including consumer products, pharmaceutics, cosmetics, transportation, energy and agriculture etc., and are being increasingly produced for a wide range of applications within industry [7]. Normally, surface sterilization of explant in plant tissue culture often uses NaOCl or HgCl2 in various concentration and time [8].

Numerous studies have demonstrated that TiO2 nanoparticles promoted photosynthesis and nitrogen metabolism and thus greatly improved growth of spinach at a concentration as low as 20 mg/l [9][10]. Another study [11][12]investigated phytotoxicology of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc and zinc oxide) on seed germination and root growth of six higher plant species (radish, rape, rye grass, lettuce, corn and cucumber). Seed germination was not affected except for the inhibition of nanoscale zinc (nano-Zn) on rye grass and zinc oxide (nano-ZnO) on corn at 2000 mg/l. Inhibition on root growth varied greatly among nanoparticles and plants. The chlorophyll contents in the sunflower seedlings supplied with magnetic nanoparticles[13].

The effect of nano-ZnO particles on the growth of plant seedlings of mung (*Vigna radiate*) and gram (*Cicer arietinum*)[14]. They found that at certain optimum concentration, the seedlings displayed good growth over control and beyond that retardation in growth was observed. Similar results were reported with the application of nano-iron oxide on soybean yield and quality. The results showed that nano-iron oxide at the concentration of 0.75 g/l was increased leaf + pod dry weight and pod dry weight. The highest grain yield was observed with using 0.5 g/l nano-iron oxide that showed 48% increase in grain yield in comparison with control [15].

The present study involve isolation of *Sargassum plagiophylum* ,characterization using UV-Vis Spectroscopy ,SEM analysis,XRD,FTIR,and evaluate the effect of silver nanopaticals in invitro seed germination, Measuring the shoot and root length and Comparing the protein profile of germinated seeds of *Vigna radiata,Vigno mungo ,Arachis hypogaea*.

## MATERIALS AND METHODS

### A. COLLECTION OF SAMPLE

*Sargassum plagiophyllum* was collected from Kovalam, Chennai, Tamilnadu. Macroalgae were brought to laboratory in polythene bags, cleaned and shade dried for about 7to 8 days. The dried samples were powdered and used further.

### B. PREPARATION OF ALGAL EXTRACT

200 mg of Algal powder were weighed and dissolved in100ml distilled water. The sample was filtered and they were used for nanoparticle synthesis.

### C.BIOSYTHESIS OF SILVER NANOPARTICLES

To the 5 ml of sample extract, 95 ml of 1mM AgNo3 solution were added and dissolved at 60°C for 15 mins in dark condition. pH and Colour change were monitored [16].

### D. CHARACTERIZATION OF BIO-SILVER NANOPARTICLES

The synthesized nanoparticles were analysed for various characteristic property like UV – Visible spectral analysis, Scanning electron microscope studies, Energy-dispersive X-ray spectroscopy analysis, X-Ray Diffraction Studies, Fourier Transform Infrared Spectroscopic analysis.

### E. IN VITRO GERMINATION RATE OF CEREALS.

**(a) Preparation of MS media (Murashige and Skoog medium)**

The commercial MS medium which is obtained contains the nutritional components, agar and sucrose. The total content is mentioned it should be weighed and allow dissolving. After dissolving it, the required amount is dispensed into culture tubes1 to 6 with replicates and kept for autoclaving. After sterilization the medium is allowed to cool and growth hormones (2, 4-D, BAP) were added to control tubes 1 and solidity in a laminar airflow hood. To the sample test tube 2, 3,4,5,6 the nanoparticles 10, 50,100,150,200ppm were added and solidify in a laminar airflow hood. The same procedure is repeated for replicates.

**(b) Surface sterilization of seeds** *(Vigna radiata,Vigno mungo ,Arachis hypogaea)*

The seeds were surface sterilized with 70% ethanol, 20% mercuric chloride solution and sterile distilled water.

**(c) Inoculation of Explants**

The surface sterilized explants were placed on the surface of MS medium and incubated at 25oC for 16h photoperiod with 250 μE/m2/ s light intensity for 2 weeks. Observe regularly for germination.

**F. MEASURING THE SHOOT AND ROOT LENGTH OF SEEDS.**

After germination, the plants were collected and measured for shoot and root length by using ruler.

**G.PROTEIN PROFILE OF GERMINATED PLANTS**

After the germination, the plants were collected from MS medium, extracted, and filtered. To the 1ml of filtrate 3 ml of Bradford reagent was added and the intensity of blue colour was read at 595 nm in a Spectrophotometer. Amount of protein was determined using bovine serum albumin served as standard.

**RESULTS AND DISCUSSION**

In this present work the biosynthesied Silver nanoparticles from *Sargassum plagiophyllum* were characterizied as the colourless solution changed to yellow colour due to the reduction of silver nanoparticles. The synthesized nanoparticles were analysed for UV – Visible spectral analysis and the peak positioned at 440nm (Fig.1), this indicated the formation of silver nanoparticles. Scanning electron microscope studies reveal the spherical shape of nanoparticles with different sizes and the average size is 440 nm (Fig.2). EDX analysis shows a small peak of silver that confirmed the presence of silver nanoparticle in the suspension (Fig.3).

**Invitro germination of seeds**

The seeds were inoculated in MS media with different 10, 50,100,150,200 ppm and germination was observed after five days. Thus the biosynthesized nanoparticle helps the plant to germinate (Fig.4, Fig.5, Fig, 6).The germination rate of *Vigno mungo ,Arachis hypogaea, Vigna radiate* at different ppm were tabulated in table 1. The bio synthesizied nanoparticles were prepared for 10, 50,100,150,200 ppm to study the germination rate. The concentrations of silver nanoparticles were chosen in the range 20, 40, 60, 80 and 100 ppm according to related studies [17].

**Measuring the shoot and root length**

After germination, the shoot and root length of *Vigno mungo ,Arachis hypogaea, Vigna radiate* were measured by using ruler and the measurement are given in the table 2. The germination rate of the seeds in MS media with biosynthesized silver nanoparticles support the growth of the plants in an in vitro condition even in the absence of plant growth harmones . And it also increases the shoot and root length of the plants.

**Protein profile of germinated plants**

The germinated plants protein profile was determined by using the fresh weight of the plants and the results were tabulated in Table 3. The protein content also increases in the plants when comparing the control plants. Application of silver nanoparticles at the concentration of 20, 40 and 60 ppm caused an increase in protein content of the two tested crop plants. The increase in protein at certain concentration suggests the optimum dose limit for the growth of common bean and corn plants. However, the decrease in protein beyond this concentration suggests the toxic effect of AgNPs. The same results were obtained by [18].

This study is to reveal the biological production of nanoparticles from marine algae posses the activity of growth promotion in plants, and it does not reveals any toxicity to the plants. Even in the absence of growth hormones the biologically synthesized nanoparticles supports the growth of the Plants.

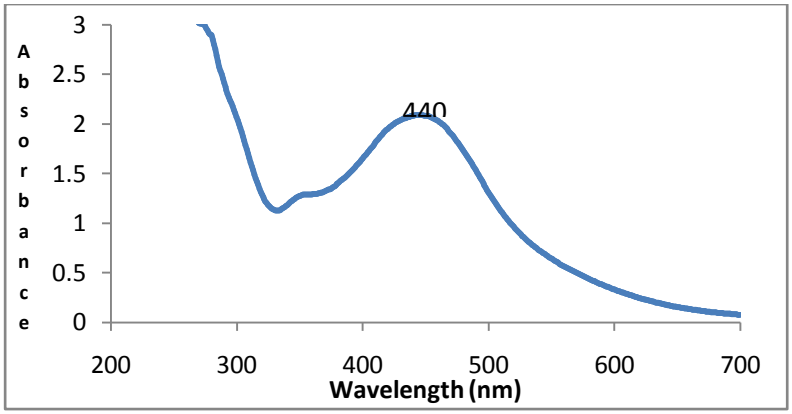


**Figure 1. Showing the UV-visible absorption spectrum of Synthesied AgNps.**

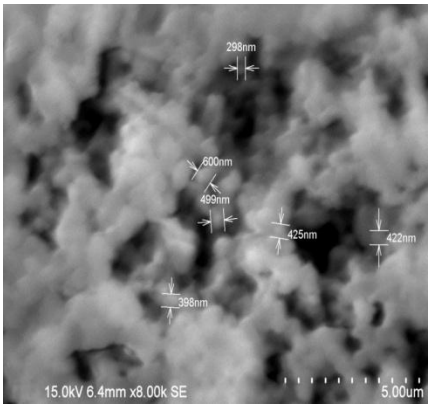


**Figure 2. Showing SEM images of Synthesied AgNps**

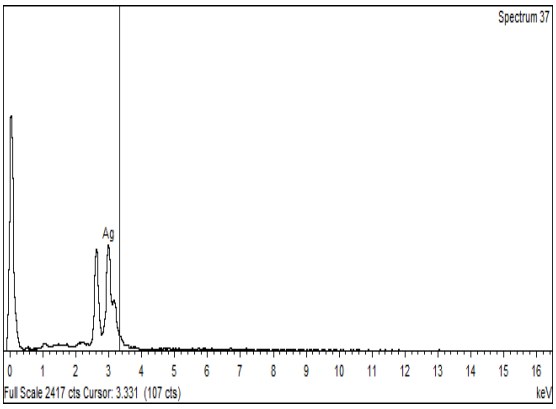


**Figure3. Showing EDX Spectra for Synthesized AgNps.**

**Figure 4.Showing the germination of *Vigno mungo* in MS media with Biosynthesied AgNPs**

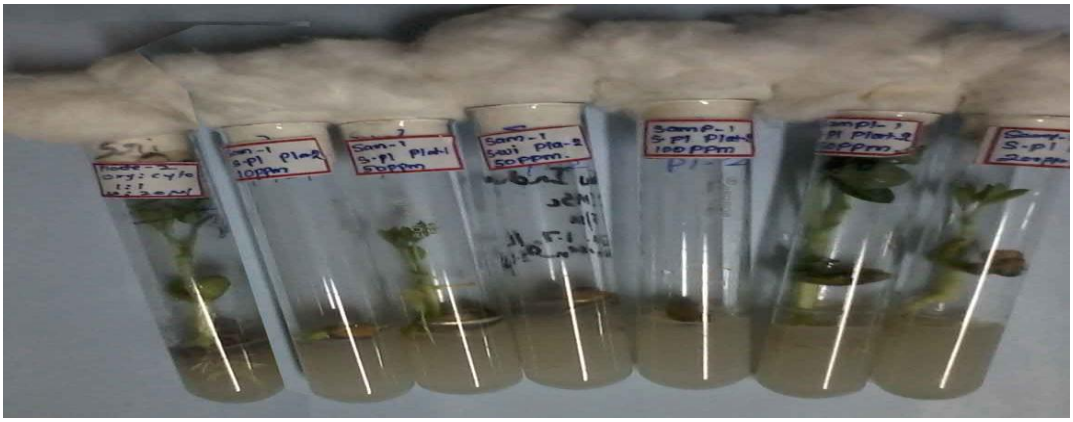

**Figure 5. Showing the germination of *Arachis hypogaea* in MS media with Biosynthesied AgNPs**

**Figure 6. Showing the germination of *Vigna radiata* in MS media with Biosynthesied AgNPs.**

**Table 1: Showing the Germination rate of** *Vigno mungo ,Arachis hypogaea, Vigna radiata*

| **Invitro study - germination of plant** | | | |
|---|---|---|---|
| **PPM** | ***Vigno mungo*** | ***Arachis hypogaea*** | ***Vigna radiata*** |
| 10 ppm | G,R/S | G,R | G,R/S |
| 50 ppm | G,R/S | G,R | G,R/S |
| 100 ppm | G,R/S | G,R | G,R/S |
| 150 ppm | G,R/S | G,R/S | G,R/S |
| 200 ppm | G,R/S | G,R/S | G,R/S |
| Control | G,R/S | G,R/S | G,R/S |

**G-germination, R-root formation, S-shoot formation**

**Table 2: Showing the Shoot and Root length of** *Vigno mungo ,Arachis hypogaea, Vigna radiate.*

| | ***Vigno mungo* (cm)** | | ***Arachis hypogaea* (cm)** | | ***Vigna radiate* (cm)** | |
|---|---|---|---|---|---|---|
| **PPM** | **Shoot** | **Root** | **Shoot** | **Root** | **Shoot** | **Root** |
| 10 | 8.0 | 1.3 | - | 0.4 | 8.5 | 0.8 |
| 50 | 7.5 | 0.9 | 2.5 | 0.5 | 8.5 | 0.5 |
| 100 | 8.1 | 1.0 | - | 0.4 | 8.0 | 0.5 |
| 150 | 7.3 | 0.9 | 6 | 1.2 | 7.1 | 0.7 |
| 200 | 5.3 | 1.0 | - | o.5 | 4.2 | 0.7 |
| control | 7.5 | 0.7 | 4.3 | 1.4 | 9.5 | 0.8 |

**Table 3: Showing the protein profile of** *Vigno mungo ,Arachis hypogaea, Vigna radiate.*

| **Treatments** | **Protein (mg/gram fresh weight)** | | |
|---|---|---|---|
| | ***Vigno mungo*** | ***Arachis hypogaea*** | ***Vigna radiate*** |
| 10ppm | 4.98 | 5.88 | 46.15 |
| 50ppm | 10.33 | 5.92 | 35.71 |
| 100ppm | 21.15 | 5.19 | 24.62 |
| 150ppm | 17.32 | 4.19 | 31.73 |
| 200ppm | 13.99 | 4.76 | 25.34 |
| control | 9.3 | 6.52 | 46.15 |

## CONCLUSION

The results of the present study demonstrating the interaction of AgNPs, synthesized using biological approach is nontoxic and environmental friendly, growth of plant in different ppm of nanoparticles does not affect the plant growth and it also helps the growth of the plants at different ppm. Even In the absence of plant growth hormones the biologically synthesized nanoparticles induces the plant growth.

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