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Effect of Nanoparticles on Seed Germination and Seedling Growth of Boswellia Ovalifoliolata – an Endemic and Endangered Medicinal Tree Taxon

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ABSTRACT

The synthesis, characterization and application of biologically synthesized nanomaterials have become an important branch of nanotechnology. In this paper, we report the synthesis of highly dispersed silver nanoparticles using a dried stem bark of *Boswellia ovalifoliolata.* Bark extract as the reducing agent and tested the effect on seed germination and seedling growth. After exposing the silver to bark extract, rapid reduction of silver ions was observed leading to the formation of silver nanoparticles in the solution. UV-VIS spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 430 nm. Scanning electron microscopy (SEM) micrograph analysis of the silver nanoparticles (SNPs) indicated that they were well-dispersed and ranged in size 30-40 nm. Biologically synthesized SNPs were employed to improve the seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic, endangered and globally threatened medicinal tree species. Four sets of seeds were germinated on Murashige and Skoog (MS) basal medium with various concentrations (10 to 30 μg/ml) of SNPs. Higher percentage (95%) of seed germination found in treated seeds when compare to control. The control seeds (water) took longer time (10 to 20 days) to sprout, whereas all treated seeds sprouted within 10 days. The maximum height (10.6 cm) observed in seedlings treated with SNPs 4 μg/ml. The possible contribution of SNPs is to facilitate the penetration of water and nutrients through seed coat and accelerate the seed germination and seedling growth of *Boswellia ovalifoliolata*.

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Keywords: *Boswellia ovalifoliolata*, endemic tree, *in vitro* germination, silver nanoparticles, seedling growth.

INTRODUCTION

Nanotechnology is a versatile field and has found applications in all most all existing fields of science. Application of nanotechnology is almost every field of science owing to the extensively research that being undertaken. Nanotechnology has the potential to revolutionize the agriculture with new tools to enhancing the ability of plants to absorb nutrients. Nanoparticles have interactions at molecular level in living cells and Nano agriculture involves the employment of nanoparticles in agriculture with the ambition that these particles impart some beneficial effects to the crop[1]. The use of nanopartcles in growth of plants and for the control of plant diseases is a recent practice[2-3]. Nanopartcles of size below 100 nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generates both positive and negative biological effects in living cell[4]. The amount of research has been increasing on the biological effects of nanoparticles on higher plants. Lu *et al.*,[5] studied the effect of mixtures of nano SiO_2 and nano TiO_2 on Soybean seed. They found that the mixture of nano particles increases nitrate reductase in Soybean increasing its germination and growth; and ZnO on growth of *Vigna radiata* and *Cicer arietinum* seedlings using plant agar method[6] and Pea nut[7].

Single walled carbon nanotubes (SWNTs) have capacity to transverse across both the plant cell wall and cell membrane[8]. Gonzales-Melendi *et al.*[9] reported that the nanoparticles act as smart treatment delivery systems in plants. Compared to plant cell walls and membranes the penetration of nanoparticles into seeds is expected to be difficult due to thick seed coat[10]. Inspite of this carbon nanotubes could effectively penetrate seed coat and influence the seed germination and plant growth[11].

Boswellia ovalifoliolata Bal & Henry is a narrow endemic and globally threatened tree species. It is deciduous medium sized tree belongs to the family Burseraceae. This tree harbours on Tirumala hills of Seshachalam hill range of Eastern Ghats of India. Tribals like Nakkala, Sugali and Chenchu used the plant and indigenous community to treat number of aliments[12]. The population density of the species had been decreasing due to several reasons. One of the reason was identified that the seeds are loosing germination capacity with in 15 days after maturation (lack of dormancy). The seeds are small with hard seed coat and exhibit less % of seed germination; and slow growth rate of seedlings[13]. Research on role of nanoparticles in higher plants needs quick attention. Eventhough several studies are concerned with the synthesis of nanomaterials using biological routes, only limited studies have been reported on the promontory effects of nanoparticles on plants in low concentration. Studies on effect of SNPs on seed germination and seedling growth were very limited. Hence in the present study an attempt has been made to test the effect of biologically synthesized SNPs on seed germination and seedling growth of *Boswellia ovalifoliolata*.

MATERIAL AND METHODS

Biogenesis of Silver nanoparticles

The stem bark of *Boswellia ovalifoliolata* was collected in the year 2009 from the Tirumala hills of Andhra Pradesh, India and were air dried for 10 days then the bark was kept in the hot air oven at 60^0C for 24 to 48 h. The dried bark was ground to a fine powder. SNPs prepared biologically using the bark of *Boswellia ovalifoliolata* following the method described by Savithramma *et al.* (14). One mM silver nitrate was added to the plant extracts separately to make up a final solution of 200 ml and centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at -4^0C. The supernatants were heated at 50^0C to 95^0C. A change in the colour of the solutions was observed during heating of process. The reduction of pure Ag^{2+} ions were monitored by measuring the UV-Vis spectrum (Systronic 118 UV- Visible Spectrophotometer) of the reaction media at 5 h after diluting a small aliquot of the sample in distilled water. Thin films of the samples were prepared on a carbon coated copper gird and SEM analysis were carried out using Hitachi S-4500 SEM Machine. The EDAX measurements of the silver nanoparticles of the bark extract were performed on Hitachi S-3400 NSEM instrument equipped with thermo EDAX attachments and the three dimensional structure with AFM (Nanosurf ® AG, Switzerland; Product: BTO2089, Vl. 3RO).

The seed germination experiment was carried out with four sets, each set with five test tubes containing MS basal medium without growth regulators. First set of test tubes consider as control which consist of MS basal medium only. Second set is MS basal medium + 1 ml of SNPs (10 μg/ml); 3^{rd} set with 2 ml (20 μg/ml) and 4^{th} set with 3 ml (30 μg/ml) of SNPs. Five seeds were placed in each test tube and observed for germination.

RESULTS AND DISCUSSION

Colour change in the solution within 10 min indicates that the reduction of silver ions into silver particles during exposure to the plant extract to $Ag(NO_3)_2$ solution. Silver nanoparticle (SNPs) exhibit dark yellowish to brown color in aqueous solution due to the surface Plasmon resonance phenomenon. The results obtained in this study are very interesting in terms of identification of potential forest plants for synthesis of nanoparticles. UV-Vis spectrograph of the colloidal solution of SNPs has absorbance peak at 430 nm and broadening of peak indicated that the particles are polydispersed. From EDAX spectrum, it is clear that *Boswellia ovalifoliolata* has recorded weight percent (39.88%) of nanoparticle followed by the SEM image showed relatively spherical shape nanoparticle with diameter ranging from 30 to 40 nm. Similar phenol-menon was reported by Chandran *et al.*,[15], Lingarao and Savithramma[16]; Ankanna and Savithramma[17].

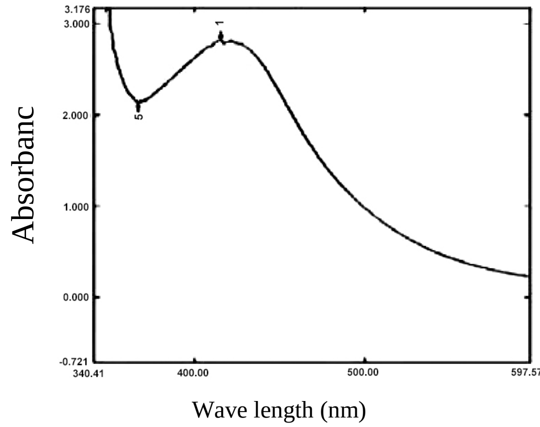
The seeds placed in MS medium containing SNPs showed 90% germination, whereas 70% germination was observed in control seeds (Table-1). The reason could be that the SNPs can penetrate through seed coat and may be activate the embryo. Khodakovskaya *et al.* (11) mentioned that the carbon nanotubes can effectively penetrate through seed coat, and influence the seed germination. Exposure of tomato seeds to Carbon nanotubes (CNTs) resulted in enhance seed germination and growth rate[10].

Sprouting of seeds was observed from 7th day onwards in SNPs treated test tubes. Whereas, the sprouting was observed on 15th day in control seeds. All seeds treated with SNPs completed the germination with in 7 to 10 days. However 10 to 20 days required for control seeds. Accumulation of SNPs were found in root cells of *Oryza sativa* due to adsorption of NPs by complex mechanism with functional groups, physical adsorption, chemical reactions with surface sites, ion exchange and surface precipitations[18]. The seedlings grown in media supplemented with SNPs increased length when compare to the control seedlings (Fig.1). Maximum height (10.6 cm) of the seedlings was found in 4th set i.e. 30 μg/ml SNPs, whereas the control seedlings are shorter than other three treatments and took more than 30 days to attain 5 cm in length. All SNPs treated seedlings attain maximum growth with in 20 to 25 days. The increased seedling growth rate may be due to the enhancing the water and nutrient uptake by the treated seeds. Tomato seeds incubated in agar medium supplemented with CNTs found that the presence of CNTs inside the seeds with high percentage of moisture[10] and nanotubes also serve as an effective nanotransporters to deliver DNA and small dye molecules into intact plant cells[8]. Mazumdar and Ahmed[18] observed higher concentrations (1000 μg/ml) of chemically synthesized silver nano-particles are toxic to the seedlings of *Oryza sativa* in Hoagland's nutrient solution. The biologically synthesized SNPs of 30 μg/ml is an optimum concentration among the selected concentrations to enhance the maximum growth in seedlings of *Boswellia ovalifoliolata* germinated in *in vitro* conditions on MS basal medium. The reason could be that the SNPs may be generated new pores on seed coat during penetration which may helps to influx the nutrients inside the seed or SNPs may carry the nutrients along with which may leads to rapid germination and growth rate. The results of the present study may be helpful to improve the % of seed germination and seedling growth in seeds especially in dormant seeds. By using this technique it can increase the amplification of plants particularly endemic trees with hard seed coat which are in the verge of extinction.

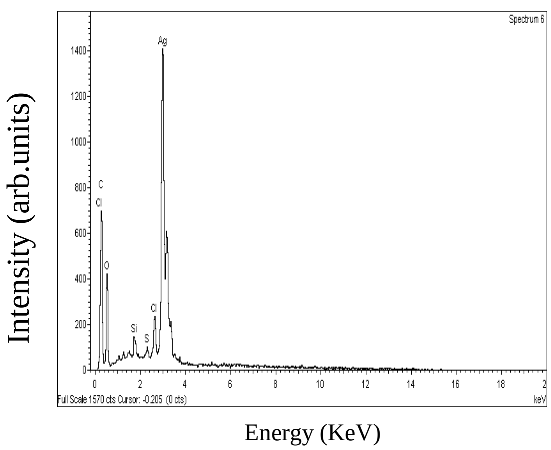
Table-1: Effect of Silver nanoparticles on *in vitro* seed germination and seedling growth of *Boswellia ovailifoliolata* values are an average of five replications ± SE.

S. No.	Concentration	% of seed germination	germination period	Seedling growth
1.	Control	70 ± 2.5	17 ± 3	3.0 ± 0.5
2.	10 μg/ml	91 ± 3.2	8.0 ± 1	5.5 ± 1.0
3.	20 μg/ ml	92 ± 2.0	8.0 ± 2	6.3±1.7
4.	30 μg/ml	95 ± 3.1	9.0 ± 2	10.6 ± 0.3

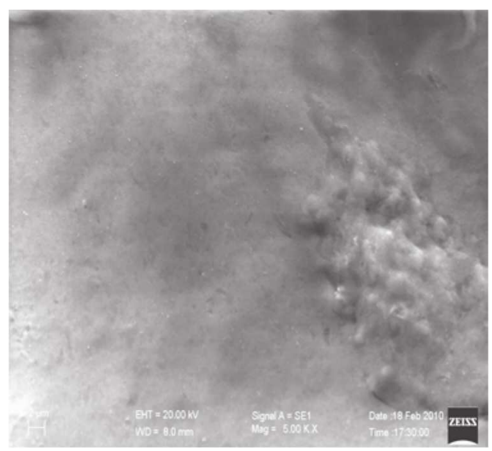
(a)



(b)



(c)



(d)

Figure 1: (a) The colour change of bark extracts,
(b) UV-VIS absorption spectra,
(c) Energy dispersive analysis of X-rays,
(d) SEM image of synthesized silver nanoparticles of stem barks of *Boswellia ovalifoliolata*

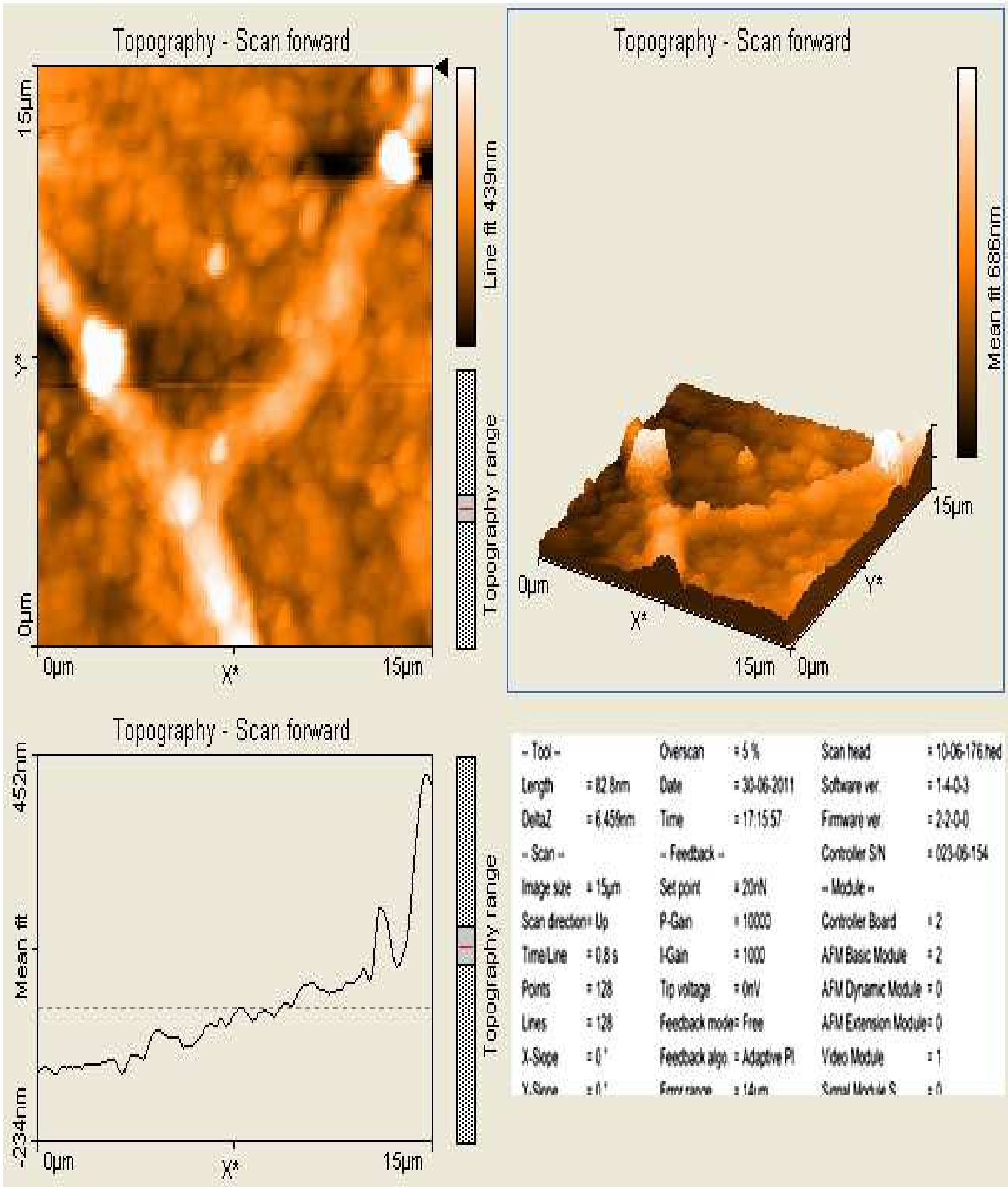


Figure 2: AFM Photograph of synthesized SNPs from *Boswellia ovalifoliolata* stem bark with 3 dimensional structures

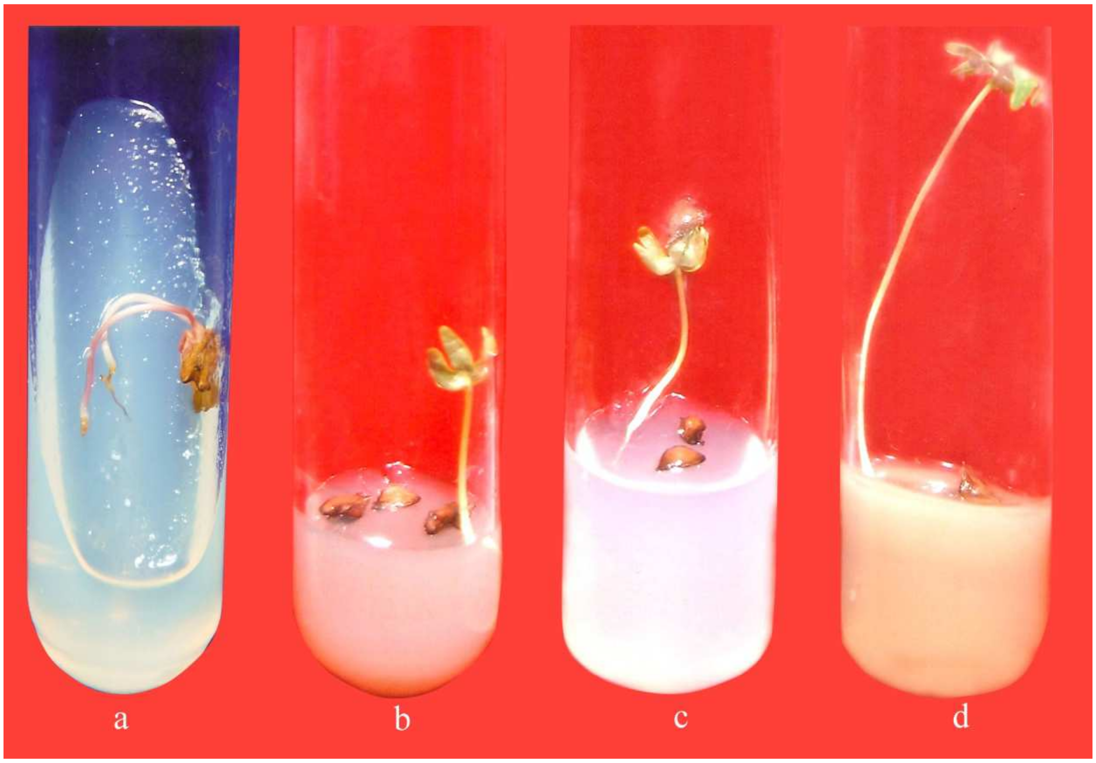


Fig.3. Effect of biologically synthesized silver nanoparticles on seed germination and seedling growth of *Boswellia ovaliofoliolata* a) Control, b) SNPs 10 μg/ml, c) 20 μg/ml and d) 30 μg/ml

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