

Effect of Silver Nanoparticles (AgNps) Produced by an Endophytic Fungus *Fusarium Semitectum* Isolated from a Medicinal Plant *Withania Somnifera* (Ashwagandha) on Seed Germination

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Abstract: AgNps were synthesized from the Endophytic fungus *Fusarium semitectum* isolated from healthy leaves of a medicinal plant *Withania somnifera* (Ashwagandha). Characterization of AgNps by UV-Vis showed a maximum absorption peak at 422nm. TEM revealed AgNps to be smaller in size, spherical in shape from 12-20nm. Different concentration of AgNps was tested against germination of Mungbean, Pigeon Pea and Chickpea seeds. Percent seed germination, root and shoot length of all the three seeds were measured. The Germination index (G.I) of Mungbean and Pigeon Pea seeds was maximum 123.33 and 117.64 with 200 μ l of AgNps and Cent percent seed germination in Mungbean seeds while it was 58.3% in Pigeon Pea. The average root and shoot length was maximum 4.6cm and 4.7cm with 100 μ l of AgNps in Mungbean whereas, shoot length was 4cm at 200 μ l and root length was 4.4cm at 100 μ l. In case of Chickpea the G.I was maximum 46.51 with maximum % seed germination of 78.5% and shoot length of 2cm with maximum root length of 4.9cm at 100 μ l of AgNps. All these results reveal that 100 and 200 μ l of AgNps can enhance root and shoot germination while, higher concentration of AgNps showed Phytotoxicity revealing that dose dependency will have good effect on overall plant growth.

1. INTRODUCTION

Application of nanotechnology is now available in various fields of science due to the extensive research being undertaken throughout the world. Nanotechnology has the potential to revolutionize agriculture with new tools to enhance the ability of plants to absorb specific required nutrients (Savithramma N et al., 2012). With the advent of nanotechnology AgNps have been applied in many fields such as medical, industrial, dairy products and also in agriculture. AgNps may be released into the environment by several routes, including during their synthesis, incorporation of AgNps into other goods, and recycling or disposal of these goods and AgNps Experts feel that the potential benefits of nanotechnology for agriculture, food, fisheries and aquaculture need to be balanced against concerns for the soil, water, and environment and the occupational health of workers. Raising awareness of nanotechnology in the agri-food sector, including feed and food ingredients, intelligent packaging and quick-detection systems, is one of the keys to influencing consumer acceptance.

There have been different and often conflicting reports on the absorption, translocation, accumulation, biotransformation and toxicity of nano particles in various plant species. Recent studies have reported that a plant's response to AgNps enhancement or inhibition of growth, depends on the AgNp dosage. Exposure to specific concentration of AgNps could enhance plant growth compared with non-exposed plants, whereas higher and lower concentrations could effect plant growth. (R. Kaveh et al., 2013 and H.Qian et al, 2013). A lot of studies have been reported on positive and negative effects of nano particles on higher plants. Due to its variable shape and size, it is difficult to predict the positive or negative effect and its mode of action in the environment and within living systems. (M.Holsapple et al., 2005)

On the basis of only a handful of toxicological studies, concerns have arisen regarding the safety of nano materials, researchers and companies will need to prove that these nanotechnologies do not have more of a negative impact on the environment. Agri-food nanotechnology is multidisciplinary in

nature. Nanotechnology application to the agriculture and food sectors is relatively recent. compared with its use in drug delivery and pharmaceuticals. Nanotechnology has the potential to protect plants, monitor plant growth, detect plant and animal diseases, increase global food production, enhance food quality, and reduce waste for “sustainable intensification”(Locke J.M. *et al*, 2000) We at our laboratory, made an attempt for employing nano particles in Agri by conducting studies on effect of AgNps on seed germination. Our results of exposure of AgNps to Mung bean, Pigeon Pea and Chickpea seeds have shown both positive and negative effect on seed germination, root and shoot growth

2. MATERIALS AND METHODS

Isolation of Endophytic Fungi

The isolation of endophytic fungus was carried out following the procedure of Ashish et al., (2015). Healthy leaves of *Withania somnifera* (Ashwagandha) were collected from Department of Botany, Gulbarga University Kalaburagi. washed several times with tap water followed by the Surface sterilization was done by immersing the leaves into a sequence of solvents finally rinsed with sterilized double distilled water, these cut leaves were dried with the help of blotting paper and inoculated onto the plates containing Potato Dextrose Agar (PDA), the inoculation was done considering that the cut surface of the leaf explant was half pierced into the medium, incubated at 28°C for 6-8 days for the growth of Endophytic fungal colonies out of the leaf segment. Fungus which grew out of the explant based on its morphological and reproductive characters using standard identification manual was identified and used for further study.

Biological Synthesis of Silver Nanoparticles

The endophytic fungus *Fusarium semitectum* was screened for the biosynthesis of AgNps. A 250 ml Erlenmeyer flask containing 100 ml of MGY medium (3g Malt extract, 3g Yeast extract, 5g Peptone, Distilled water 1000 ml) was incubated at 28°C for 2 days in static position. The fungal mycelia obtained was separated by filtering through What man filter paper No.1 and then repeatedly washed with distilled water Further, the biomass was again suspended into the flask containing double distilled water and harvesting the fungal filtrate through What man filter paper No 1, which was then treated with 1mM Silver nitrate (AgNO_3) solution and incubated at 28°C for reduction (Ag^+ to Ag^0) and observed for the color change from pale white to brown, which is an indication for the production of AgNps

Characterization of Silver Nanoparticles

UV-Vis Spectroscopy

The synthesized AgNps solution was observed for the change in color visually of the mycelia free filtrate when treated with 1mM silver nitrate solution and was confirmed by sampling the reaction mixture at regular intervals and the absorption maxima was scanned by UV-Vis spectra at the wavelength between 350 to 500 nm.

Transmission Electron Microscopy (TEM)

The morphology of biosynthesized AgNps was studied using TEM technique (Hitachi H-7500). A drop of biosynthesized AgNps solution was placed on a carbon coated copper grids and kept overnight under vacuum desiccation and dried. The sample was loaded onto a specimen holder. Transmission electron micrographs of samples were taken.

Seed Germination Experiment

Mungbean, Pigeon Pea and Chickpea seeds were collected from local market of Kalaburagi city, Karnataka. These seeds were several times washed with tap water to remove the dirt and adherent particles, surface sterilized followed by washing with distilled water then some of the seeds were dipped in distilled water (Control) and in the AgNps solution of 3 different concentrations like 100, 200 and 300 μ l. The seeds were treated with the above mentioned concentrations and subsequently transferred to petridishes containing one piece of filter paper about 6-7 seeds were evenly placed in the petridishes and were allowed to germinate on lab bench at room temperature. After 5 days of growth the root and shoot length were measured and recorded. The germination index and % seed germination were determined by using the formula given below

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$$\% \text{ Seed Germination} = \frac{\text{No. of germinated seeds}}{\text{No. of Inoculated seeds}} \times 100$$
$$\text{Germination Index (G.I)} = \frac{\text{Average Root length}}{\text{Average Shoot length}} \times 100$$

3. RESULTS AND DISCUSSION

Isolation of Endophytic Fungi and Biosynthesis of AgNps

Endophytic fungus was grown out of the leaf segment of *Withania somnifera* (Ashwagandha) after 7 days of incubation on PDA plates. The fungus was subcultured and identified as *Fusarium semitectum*. based on the morphological and microscopic observations at Agharkar Research Institute, Pune. The Endophytic fungus *Fusarium semitectum*, isolated was used for the production of AgNps. The extracellular filtrate was treated with 1mM AgNO₃ solution and the reduction was indicated by the change in color from pale white to brown indicating the production of AgNps and later was confirmed by UV-Vis spectrophotometric analysis (Fig 1a & b)

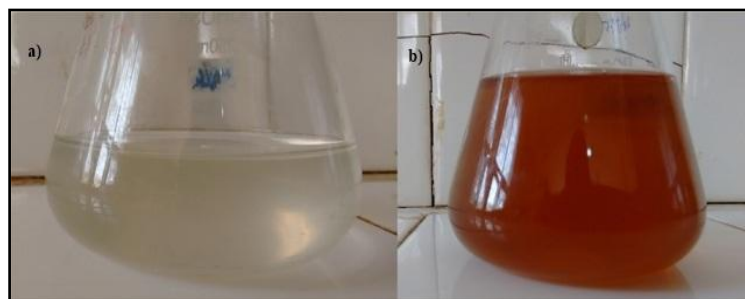


Fig 1a. Enzyme filtrate of Endophytic fungus, *Fusarium semitectum*.

Fig 1b. Treatment with 1mM silver nitrate solution changes the color to reddish brown

UV-Vis Spectroscopy

UV-Vis spectrophotometric analysis was carried out for the AgNps produced using Endophytic fungus *Fusarium semitectum*, with the maximum absorbance peak at 422 nm was observed (Fig 2). The values lie between 300 to 500 nm which confirms the production of AgNps. Chandrasekhar *et al.*, (2014) reported the production of AgNps from the fungal extract of the Endophytic fungus, *Penicillium nodositatum* with the maximum absorbance peak at 420 nm, Dattu *et al.*, (2013), reported the production of AgNps with the maximum absorption peak by Surface Plasmon Resonance (SPR) at 420 nm from the fungal extract of *Penicillium sp.*, an Endophytic fungus isolated from *Curcuma longa* (turmeric). Similarly other researchers Shivaraj *et al.*, (2014) also reported single peak with a maximum absorbance at 416 nm corresponding to the SPR of AgNps. Our results correlate with the above said authors.

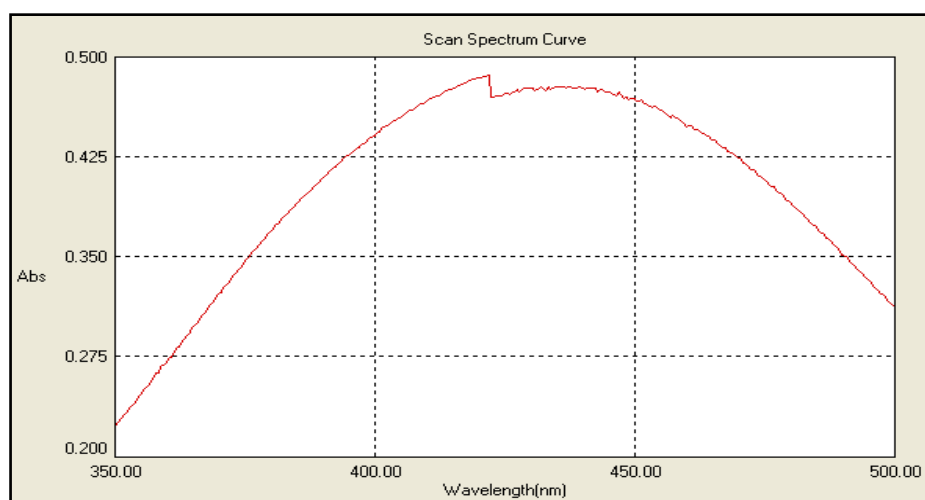


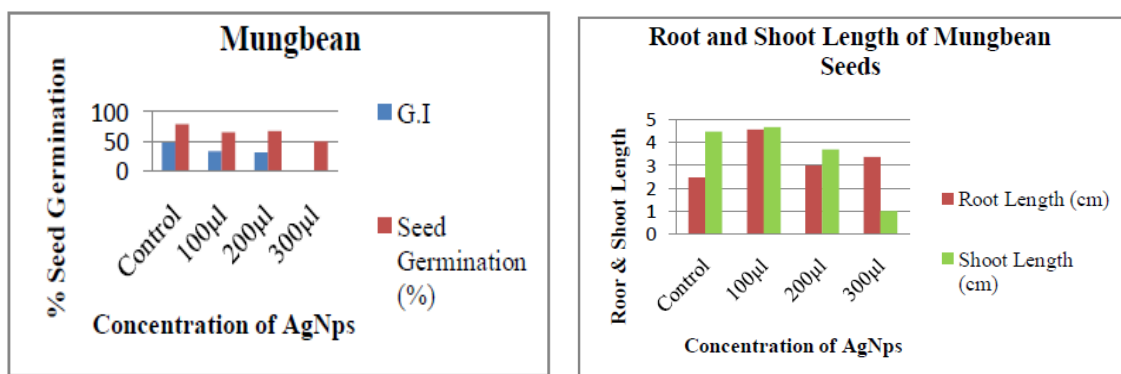
Fig 2. UV-Vis spectra of AgNps synthesized by the Endophytic fungus *Fusarium semitectum*

Transmission Electron Microscopy (TEM)

TEM determines the morphology and size details of the synthesized AgNps. Size and shape of the nanoparticles were recorded and found to be well dispersed, small in size and spherical in shape ranging from 12-20 nm (Fig 3). Prema *et al.*, (2014), reported spherical shaped AgNps with the size ranging from 20 to 35nm produced by extracellular filtrate of *A.terreus* and Dattu *et al.*, (2013), reported well dispersed spherical shaped nanoparticles with a size range of 25-30 nm using extracellular filtrate of Endophytic fungus *Penicillium sp.*, Kirthika *et al.*, (2014) reported the spherical shaped AgNps with a size approximately 25 nm from aqueous plant extract of *T.chebula*. Surprisingly the size of the nanoparticles produced by our organism appears to be the smallest, which may show good effects in various fields.



Fig 3. TEM showing well dispersed AgNps by Endophytic fungus *Fusarium semitectum*.

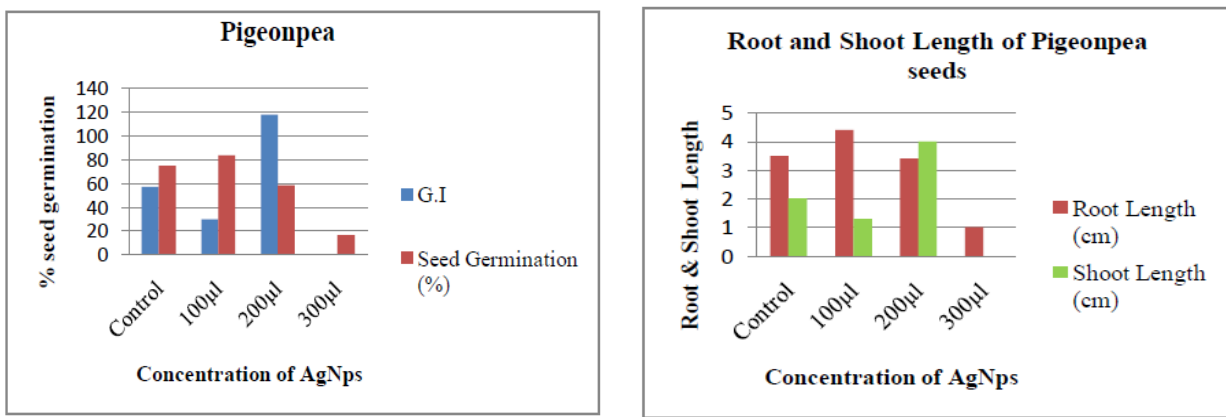


Graph 1 & 2. Seed Germination (%), Root and Shoot index of Mungbean Seeds



Fig 4. Germinated Mungbean Seeds treated with different concentrations of AgNps

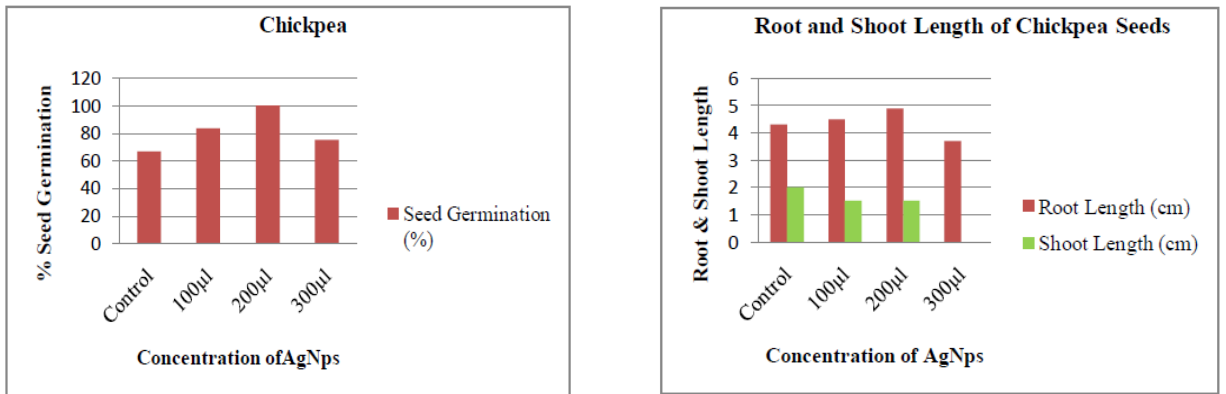
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Graph 3 & 4. Seed Germination (%), Root and Shoot index of Pigeonpea Seeds



Fig 5. Germinated Pigeonpea Seeds treated with different concentrations of AgNps



Graph 4 & 5. Seed Germination (%), Root and Shoot index of Chickpea Seeds



Fig 6. Germinated Chickpea Seeds treated with different concentrations of AgNps

Seed Germination Experiment

From the figures the Percent (%) seed germination, growth index, root and shoot germination in cms were calculated. In case of Mungbean, Pigeon Pea and Chickpea seeds were analysed. The results were so encouraging that from the figures it is clearly evident that AgNps have shown very good effect on the 5th day of germination.

Effect of AgNps on Mungbean Seeds: The G.I index of Mungbean seeds were maximum 123.33 with 200 μ l of AgNps while with same concentration % seed germination was cent percent whereas the average root and shoot length were maximum, i.e 4.6cm and 4.7cm respectively with 100 μ l of AgNps concentration (Fig 4, Graph1&2)

Effect of AgNps on Pigeon Pea: The G.I index of pigeonpea were again maximum 117.64 with 200 μ l of AgNps which was same as that of Mungbean seeds while, the % seed germination was 58.3% when root length and Shoot length were compared the shoot length was maximum 4.0cm at 200 μ l while, the root length was maximum 4.4cm at 100 μ l which reveals that a concentration between 100 and 200 μ l of AgNps are not toxic beyond the concentration of 200 μ l i.e 300 μ l the germination index was 'nil' and the % seed germination index was minimum i.e 16.6 and with minimum root length of 1cm and no shoot length at all (Fig 5, Graph 3&4)

Effect of AgNps on Chickpea: Similarly, the Chickpea results depicts that though the G.I was maximum i.e 46.51 with maximum % germination of 78.5 and shoot length of 2cm but amongst the concentration of AgNps used 100 μ l showed a maximum G.I of 33.33 with the % seed germination of 66.6% with maximum root length of 4.9cm even higher than the control. The shoot length of 1.5cm each was observed in both 100 & 200 μ l of AgNps whereas the G.I and the shoot length was 'nil' with 300 μ l showing lowest root length of 3.7 with lowest % seed germination of 50%. (Fig 6, Graph 5&6)

Lifen wang et al., (2015) reported the germination rate of *R.sativus* ses ranged from 90 to 97% and no significant difference between control, AgNps treatment. They reported that AgNps do not have accurate toxic effect on *R.sativus* seed germination. While Lin & xing (2007) showed that nanoparticles had less effect on seed germination compared to seedling growth in *R.sativus*

The increasing use of nano particles in daily products is a great concern, particularly when the positive and negative impacts of nano particles on nano particles on environment are not known Zainab et al., (2015) investigating the impact of AgNps reported that the highest germination % (73.33%) and the highest germination rates (1.59 seeds/day) for watermelon were recorded at 2mg/ml AgNps for zucchini plants highest germination % (86.67 & 90%) and highest germination rates (1.68 & 1.66 seeds/day) were recorded at 0.5 & 2.5mg/ml of AgNPs while in corn seeds the highest germination rate was 6.5 seeds/day with 1.5mg/ml of AgNps. They reported that among three plant sps. Zucchini seeds, the germination time of which increased significantly at 1.5 and 2.5mg/ml of AgNps translating to later germination than untreated plants.

Reports reveal that it is probable that nanoparticles penetrate the seed coat and exert a beneficial effect on the process of seed germination by increasing water absorption by seeds (zheng et al., 2005). They also increase nitrate reductase enzyme levels, increase the seed abilities to absorb and utilize fertilizer, promote seed antioxidant systems (Lu et al., 2002), reduce oxidative stress by reducing H₂O₂ superoxide radicals and malonyldialdehyde content and increase the activities of some enzymes such as superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase All these studies express that the biocompatibility of AgNps can be determined and the potential agricultural applications for nanoparticles in crop improvement and food production can be judged.

4. CONCLUSION

Use of nanoparticles is leading to the progress of a range of inexpensive applications for enhanced plant growth. Applications of nanomaterials can encourage earlier plant germination and improve plant productivity. (Reyhanch azimi et al., 2013). AgNps have been known as a kind of antibiotics. These particles provide protections against fungi and bacteria present in the seeds germination rate, root and shoot elongation as a rapid phytotoxicity test method posses several advantages such as sensitivity, cost effectiveness and stability for unstable chemicals our results revealed that a higher concentration of 300 μ l had no significant effect, rather showed toxic effect on both root and shoot germination while a concentration of 200 μ l increased the rate of germination compared to control hence AgNps not only can be used as test material to reveal their non toxicity mechanism in plants and genetic imbalance but also for determining their biocompatibility and identifying their potential agriculture applications in crop improvement and food productivity.

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