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Invitro Efficacy of Biosynthesized Silver Nanoparticles on *Trigonella foenum-graecum* L. Seed Germination and Growth

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Nanoparticles have been suggested as a relatively new hope in agriculture area as a biofertilizer to make agriculture more sustainable. The purpose of this study was to compare effect of biologically synthesized silver nanoparticles on growth and development of plant during germination as well as seedling and vegetative period. Fertilizers are an important key factor for the production of any crop in both developed and developing countries. Nanoparticles as biofertilizer are a relatively new approach in agriculture area and it has had many promising results. It has potential contribution in slow release of fertilizers. In the present investigation, Silver nanoparticles were biologically synthesized using *Aspergillus terreus* and *Aspergillus niger* strains collected from culture center in aseptic conditions. It was observed that low concentrations of Ag nanoparticles being 20 ppm, 40 ppm and 60 ppm showed better growth than higher concentrations like 80 ppm and 100 ppm. The plants treated with low concentrations of Ag nanoparticle showed better growth than the control. So, to conclude there is significant difference in the growth of plants which had been treated with Ag nanoparticle and those without.

Keywords: *Aspergillus terreus*, *Aspergillus niger*, Biosynthesis, Biofertilizer

Introduction

Nanoparticles (NP) are group of atoms in the size ranges from 1–100 nm. Many researchers investigated different metallic nanoparticles such as Gold and Silver NPs.[1,2] AgNPs possess low toxicity on human cell and good biocompatibility, low volatility and high thermal stability.[3,4] To date, physical, chemical and biological reduction approaches were engaged in the synthesis of metallic AgNPs.[5,2] Further, if those methodologies have been practiced in production of nanoparticles, a chemical agent toxicity effect has a worry in environmental policy. Unlike physical and chemical processing, biological synthesis is further favored in which the biological system provides an innovative research idea in nano scale materials production.[6] Although several bacteria have been used in the biological synthesis of AgNPs, the eukaryotic fungal kingdom has been known to secret higher amount of bioactive compounds which made fungi more suitable in large scale biomass production in nano factories[7] and also the extracellular biosynthesis using fungi could also make down processing much easier than bacterial biosynthesis of nano silver particles. For instance, *Fusarium oxysporum* and *Verticillium* species[8], *Aspergillus fumigatus*[9], *Aspergillus niger*[10], and *Penicillium fellutanum*[11] fungal spp. have been used commonly in these days for microbial production of silver nanoparticles. Here we investigated the biosynthesis of AgNPs using *Aspergillus terreus* and *Aspergillus niger* and its effect on the morphology of the plant during its germination and vegetative period.

Materials and Methods

Sample Collection and Biomass Preparation

In the present investigation, *Aspergillus terreus* and *Aspergillus niger* sample were collected from MTCC, IMTECH, Chandigarh. *A. terreus* was grown in Potato Dextrose Broth *Himedia/* M403-100G for the preparation of biomass. Inoculation of flasks with spores was done and incubated at 28°C on a rotary shaker (120 rpm) for 96 h. Further harvesting of biomass was done by filtration through what man filter paper No. 1 and the crude cell filtrate was recovered for further experimentation.[12]

Biosynthesis and Purification of Biosynthesized AgNPs

AgNPs were synthesized by drop wise addition of 50 mL cell extract (filtrate) into 10 mL $AgNO_3$ solution (10 mmol/L) in a 250 mL Erlenmeyer flask and then incubated at 28°C in dark for 24 h. A flask with no addition of silver ion was used as control.[13] Synthesized nanoparticles were purified by

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centrifugation (5000 rpm for 30 minutes) followed by 3 times washing with double distilled water to make the solution contamination free and dried in hot air oven at 55–60°C for 3 hrs. Purified nanoparticles were then stored at 4°C till further use.[2]

Pre-treatment of Seeds

Treatments included: 20, 40, 60, 80 and 100 ppm of SNPs and only distilled water served as control. The seeds of Trigonella were soaked in different dilutions of Ag nanoparticles synthesised from *A. Terreus* and *A. niger* respectively. The seeds were checked for germination time and observed for the growth and length of the shoot after 3 days. After the seeds had geminated, those were kept in pots containing equal amount of soil. Each pot was treated with different dilutions of Ag nanoparticles from both *Aspergillus terreus and Aspergillus niger* except control. It was watered everyday and checked for growth.[14]

Results and Discussion

Biological synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized from *A.terreus* and *A.niger* (Fig. 1) and then, purified before treatment with seed by using centrifugation (5000 rpm for 30 minutes). Earlier similar biological synthesis was also reported through plants[2] and remarkable antibacterial efficacy of biologically synthesized silver nanoparticles was reported in our earlier similar study.[15]

Pre Treatments of Seeds and Effect of Biosynthesized AgNPs on Germination and Growth of Plant

Treatments included: 20, 40, 60, 80 and 100 ppm of SNPs and only distilled water served as control. The seeds of *Trigonella* were soaked in different dilutions of Ag nanoparticles synthesised from *A. terreus* and *A. niger* respectively. During the germination period, results were obtained on the shoot length and width of the seedlings as shown in Fig. 2. Seedling growth was studied after pre treatments as shown in Table 1. After the germinated, seeds were placed in pots containing soil with different ppm of Ag- nanoparticles and a control with no Ag-nanoparticles (Fig. 3). In an earlier study NPs tested in investigation, which found supportive in enhancing seed germination of sorghum and cowpea seeds.[14] A lot of ambiguity exists in the research on effect of

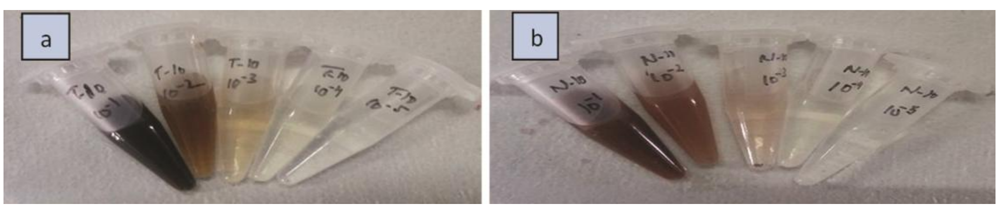

Fig. 1 — (a) Ag nanoparticles of different dilutions synthesized from *A. terreus;* (b) Ag nanoparticles of different dilutions synthesized from *A. niger*

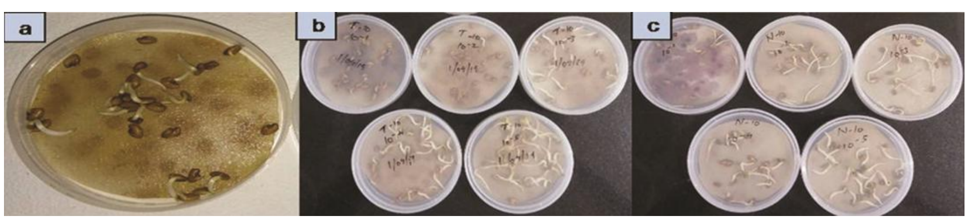

Fig. 2 — Seed treatment with biosynthesized AgNPs: (a) - Control (b) - Seed treated with AgNP's from *A. terreus*, (c) - Seeds treated with AgNP's from *A.niger*

Fig. 3 — Seed treated with AgNP's: Control, (b) from *A.niger* (c) from *A.terreus*

Table 1 — Comparison of seedling growth after pre-treatment (H- Height of plant; N- Number, L- length, and W-width of leaf)

S. no		Control	Ag-NP's synthesized from *Aspergillus terreus* (ppm)					Ag-NP's synthesized from *Aspergillus niger* (ppm)				
			20	40	60	80	100	20	40	60	80	100
1	H	3	3.8	3	2.9	0	0	3	3.3	2.8	2	3
	N	4	7	5	6	0	0	6	6	6	6	2
	L	3	1.3	1.5	1.3	0	0	1.4	1.5	1.5	1.4	1.5
	W	0.3	1	1	0.9	0	0	0.8	0.5	0.7	0.9	0.5
2	H	1.5	4	3.6	2.7	0	0	4	4.1	3.5	0	0
	N	3	6	4	5	0	0	6	6	6	0	0
	L	1.6	2.1	2	1.8	0	0	2.5	2.1	2	0	0
	W	0.4	0.8	1.1	0.26	0	0	0.9	0.6	0.3	0	0
3	H	2	3.9	3	3.4	0	0	3.8	4	3.2	1	2.5
	N	6	8	6	3	0	0	6	6	2	4	6
	L	0.8	2.2	1.7	1	0	0	2.4	1.8	1.4	1.3	0.4
	W	0.5	0.7	0.3	0.42	0	0	1	0.8	0.6	0.6	0.2
4	H	3	3.5	2.8	3	0	0	4.2	3.4	2	3.1	2
	N	4	6	6	6	0	0	6	5	6	6	3
	L	1	1.8	1.5	1	0	0	1.7	1	1.3	1.4	1.1
	w	0.7	0.6	0.45	0.5	0	0	0.4	0.5	0.6	0.2	0.35

NPs on germination of seeds. Some of researchers reported negative effect of NPs on seed germination.[14] In one earlier similar study effect of metal nanoparticles on germination of *Sorghum* grain was investigated and negative effect was observed i.e. growth was decreased significantly.[16] In positive aspect, ZnO NPs have shown better effect of seedling growth in cluster bean and tomatao.[17] In other study strong effect of 6-nm gum coated silver nanoparticles on growth and germination of wetland plants was demonstrated in comparison to PVP coated nanoparticles.[18] In our study significant growth of plant was observed when treated with AgNPs.

Conclusions

The results obtained from the above experiment demonstrate that low concentrations of biosynthesized AgNP's shows a significant difference in the growth and development of plant during the germination as well as seedling and vegetative period. Observation of the plants during the seedling and vegetative stage showed vibrant growth in low concentrations of AgNP's with larger number of leaves and longer shoots. From the present study it was observed that low concentrations of Ag nanoparticles being 20 ppm, 40 ppm and 60 ppm showed better growth than higher concentrations like 80 ppm and 100 ppm. So, to conclude that there is remarkable difference in the growth of plants with and without treatment of Ag nanoparticle. Among the plants treated with nanoparticle, lower doses or ppm of Ag nanoparticle showed better growth than those with higher ppm.

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