Effect of Nano Silver on Seed Germination and Seedling Growth in Fenugreek Seed

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Abstract—**We have investigated the effects of silver nanoparticles on plant growth parameters such as Root length, fresh weight, Dry weight, Speed of germination and % Germination contents of economic important pulses, Fenugreek (*Trigonella foenum-graecum*). The study was carried out in a randomized block design with three replications. Five levels of silver nanoparticles (0, 10, 20, 30 and 40μg mL^{-1}) were used. After germination, daily supply with 15ml from each concentration was carried out for 12 days during plant growth. Seed germination results indicate that AgNPs at their lower concentrations promoted seed germination and early seedling growth in Fenugreek, however at higher concentration showed slight adverse effects. Additionally, the lowest amount of these parameters was found with control plants, but the enhancing level of silver nanoparticles resulting in the reduction of these compounds. A significant positive influence on root length, root fresh weight and root dry weight root elongation was observed for all seeds in compared to those of unexposed control germination. The results showed that the effect of AgNPs was significant on germination percentage in P≤0.05. The results of this experiment showed that the use of AgNPs increased the germination in Fenugreek.**

Index Terms—**nanotechnology, silver, Fenugreek, seed germination**

I. INTRODUCTION

Nanotechnology is a promising field of interdisciplinary research. It opens up a wide array of opportunities in various fields like medicine, pharmaceuticals, electronics and agriculture.

The potential uses and benefits of nanotechnology are enormous. Nanoparticles (Nano Scale Particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100nm [1], [2], that can drastically modify their physico-chemical properties compared to the bulk material [3].

The majority of the reported studies point to the positive impacts of nanoparticles on plant growth with a few isolated studies pertaining to negative effect. In order to understand the possible benefits of applying nanotechnology to agriculture, the first step should be to analyze penetration and transport of nanoparticles in plants. 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 20mg/l [4]-[6].

Nanoscale titanium dioxide (TiO2) was reported to promote photosynthesis, and growth of spinach [7].

Another study by Mahajan *et al.* [8] studied the effect of nano-ZnO particles on the growth of plant seedlings of mung (*Vigna radiata*) and gram (*Cicer arietinum*). They found that at certain optimum concentration, the seedlings displayed good growth over control and beyond that retardation in growth was observed. Silver nanoparticles (AgNPs) are currently one of the most widely commercially used nanomaterials [9].

Silver ions such as AgNPs have been recognized to inhibit ethylene action [10]. This effect of silver ions on ethylene was reported by several researchers [11].

Silver eliminates unwanted microorganisms in farmer soils and hydroponics systems. It is being used as foliar spray to prevent fungi, rot, moulds and several other plant diseases. Moreover, silver is a great plant-growth stimulator, including silver salt, silicate and water soluble polymer to radioactive rays [12].

Nano silver or silver nanoparticles, is one of the most commonly used in the field of nanoparticles after carbon nanotubes that every day is added in its application to the Nano world.

Silver nanoparticles mainly due to physical and chemical properties of that particular show in their use of electronic, optical, biochemical and pharmaceutical and health are frequently used. Seed germination is an important phenomenon in modern agriculture because it is a thread of life of plants that guarantee its survival.

The recent advances in nanotechnology and its use in the field of agriculture are astonishingly increasing; therefore, it is tempting to understand the role of silver nanoparticles (AgNPs) in the germination of seeds.

Fenugreek (Trigonella foenum-graecum) is an annual plant in the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It is cultivated worldwide as a semiarid crop, and its seeds are a common ingredient in dishes from the Indian subcontinent.

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Major fenugreek-producing countries are Afghanistan, Pakistan, India, Iran, Nepal, Bangladesh, Argentina, Egypt, France, Spain, Turkey, and Morocco. The largest producer is India, where the major producing states are Rajasthan, Gujarat, Uttarakhand, Uttar Pradesh, Madhya Pradesh, Maharashtra, Haryana, and Punjab. Rajasthan accounts for over 80% of India's output [13].

Fenugreek is used as an herb (dried or fresh leaves), spice (seeds), and vegetable (fresh leaves, sprouts, and microgreens). Sotolon is the chemical responsible for fenugreek's distinctive sweet smell.

The first recorded use of fenugreek is described on an ancient Egyptian papyrus dated to 1500 B.C. Fenugreek seed is commonly used in cooking. Historically, fenugreek was used for a variety of health conditions, including menopausal symptoms and digestive problems. It was also used for inducing childbirth. Today, fenugreek is used as a folk or traditional remedy for diabetes and loss of appetite, and to stimulate milk production in breastfeeding women. It is also applied to the skin for inflammation [14].

Fenugreek contains high concentrations of choline, tryptophan, ascorbic acid, niacin and potassium. Choline is important for athletic performance, tryptophan is a serotonin precursor, ascorbic acid is a powerful antioxidant 3, and niacin and potassium are critical for immune function [15].

In view of the available literature, the present experiment was designed to investigate the effect of AgNPs on the characteristics of germination of Fenugreek (*Trigonella foenum-graecum*) seed.

II. Materials and Methods

This study was carried out in a randomized complete block design with three replications in the Biotechnology lab, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad. The AgNPs were obtained from US Research Nanomaterial's, Inc. Transmission Electron Microscopy (TEM) images of silver nanoparticles with diameters of 20nm, shown in Fig. 1.

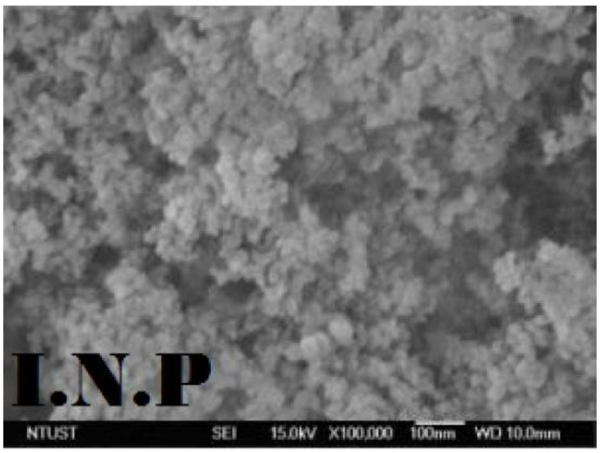

Figure 1. Silver nanopowder (Ag, metalbasis)

True density: 10.5g/cm^3, Purity: 99.99%, APS: 20nm, SSA: ~18-22 m^2/g, color: black morphology: spherical

A. Chemicals

All the chemicals were procured from Sigma Aldrich, USA. The physical characteristics of the nanoparticles as reported by the suppliers are as follows: size of the particle (near 20nm), surface area (5.0m^{2}g^{-1}) and density (10.5g/cm^3).

B. Preparation of Nanoparticle Dispersion

The SNPs were suspended in deionized water and were dispersed using ultrasonic vibrations (100W, 30kHz) for 30 min to produce four different concentrations, (0, 10, 20, 30 and 40μg mL^{-1}). All SNPs concentrations were selected arbitrarily.

C. Seed Experiment

Seeds of Fenugreek (Trigonella foenum-graecum) were used for the study and purchased from local market (Fig. 2). The seeds were stored in the dark under room temperature. All the seeds were first checked for their viability by suspending them in deionized water. The seeds which settled to the bottom were selected for further study. Seeds were sterilized in a 5% sodium hypochlorite solution for 10 minutes [16], rinsed through with deionized water several times.

Figure 2. Fenugreek-Seeds

After surface sterilization, the seeds were rinsed in deionized water thrice and were then stirred for 2 h in SNPs dispersion (0, 10, 20, 30 and 40μg mL^{-1}) using a magnetic stirrer. Whatmann No.1 filter paper was then placed into each Petri dish (100mm×15mm) and 5ml of the respective particle suspensions were added using a Pasteur pipette. The seeds were then transferred to the Petri dish, with 25 seeds per dish at controlled temperature of 25±1 ℃.

Silver nanoparticles in different concentration (0, 10, 20, 30 and 40μg mL^{-1}) were prepared directly in deionized water and dispersed by ultrasonic vibration for one hour. Each concentration was prepared in three replicates.

Every other day supply with 15ml silver nanoparticles per every test plantlets was carried out for 14 days along with control. After 14 days of growth, the shoot and root lengths were long enough to measure using a ruler.

The controlled sets for germinations were also carried out at the same time along with treated seeds. Germinated seeds counted daily for 14 days in lab and Germination Percentage (GP) were calculated in the last day.

D. Germination Speed Index (GSI)

Conducted concomitantly with the germination test, with daily calculation of the number of seeds that presented protrusion of primary root with length ≥2mm, always at the same time during the trial.

The germination speed index was calculated by the sum of the number of seeds germinated each day, divided by the number of days elapsed between the seeding and germination [17], according to the Maguire formula (1).

$$GSI = \frac{G1}{n1} + \frac{G2}{n2} + \cdots + \frac{Gi}{ni}$$

where:
GSI = seedlings' germination speed index;
G = number of seeds germinated each day;
N = number of days elapsed from the seeding until the last count.

E. Fresh and Dry Mass

The fresh mass was quantified through weighing in precision scale, and the dry mass was determined through weighing in a precision scale after permanence of the material in a kiln with air forced circulation, at a temperature of 70 ℃, until constant weight. In the end of experiment, radical and plumule length and fresh weight measured. Plants were placed in oven at 70 ℃ for 48h and weighted with sensitive scale.

F. Statistical Analysis

Statistical analysis each treatment was conducted with three replicates and the results were presented as mean ± SD (Standard Deviation). The results were analyzed by one way Anova with used Minitab Version 16.

III. Results and Discussion

Nanotechnology has emerged as a new discipline, and nanoparticles have become a Centre of attraction for researchers because of its unique physico-chemical properties compared to their bulk particles [11] and Nanotechnology is considered as one of the possible solutions to problems in food and agriculture.

As we know seed germination provides a suitable foundation for plant growth, development and yield. In present investigation, different concentration (0, 10, 20, 30 and 40μg mL^{-1}) of AgNPs were prepared in distilled water and used for the treatment in Fenugreek seeds to study their effect on seed germination and early seedling growth.

A significant positive influence on root and shoot elongation was observed for all seeds in compared to those of unexposed control germination. Seed germination results indicate that AgNPs at their lower concentrations promoted seed germination and early seedling growth in Fenugreek, however at higher concentration showed slight adverse effects.

The results showed that the effect of AgNPs was significant on germination percentage in P≤0.05. Mean comparison showed that the highest germination percentage (78%) was achieved by use of 20μg mL^{-1} of AgNPs. The results of this experiment showed that the use of AgNPs nanoparticles can increased the germination in Fenugreek.

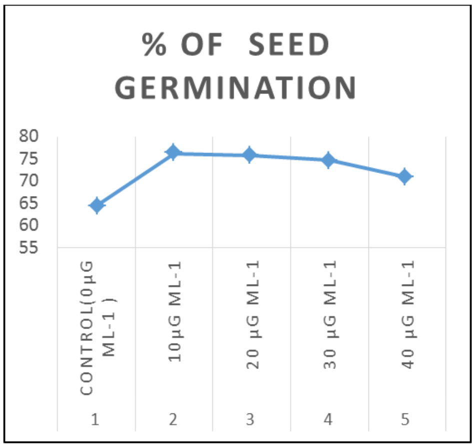

Figure 3. Effects of silver nanoparticles on germination percentage of Fenugreek (The values are the means of three replicates), (1=0 μg mL^{-1}, 2=10 μg mL^{-1}, 3=20 μg mL^{-1}, 4=30 μg mL^{-1} and 40 μg mL^{-1})

Among the treatments, application of 10μg mL^{-1} of Nano silver proved best by giving the highest values for percent seed germination, germination mean time, seedling vigor index and seed germination index. In the present experiment application of Nano silver enhanced seed potential by increasing the characteristics of seed germination (Fig. 3).

Root systems were well recognized as they were fully germination in treated experiments in less time as compared to control. The results revealed that the level of seed germination and subsequent growth of those seedlings that germinated were both decreased with increasing concentrations of AgNPs (Fig. 4).

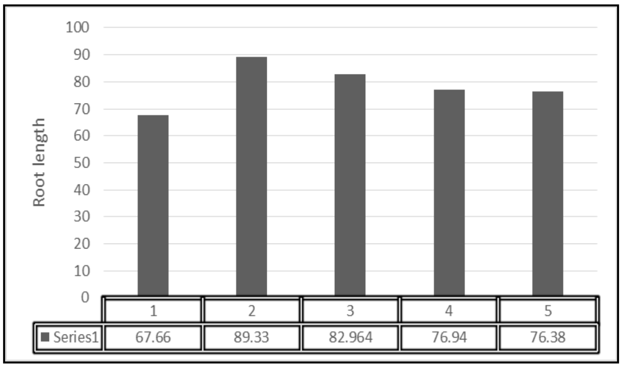

Figure 4. Effects of silver nanoparticles on root length of Fenugreek (The values are the means of three replicates), (1=0 μg mL^{-1}, 2=10 μg mL^{-1}, 3=20 μg mL^{-1}, 4=30 μg mL^{-1} and 40 μg mL^{-1})

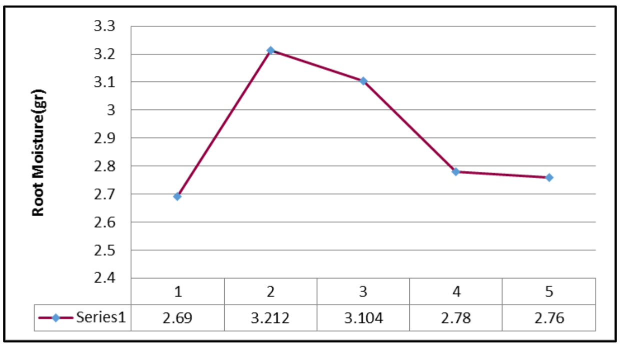

Figure 5. Effects of silver nanoparticles on root moisture of Fenugreek (The values are the means of three replicates), (1=0 μg mL^{-1}, 2=10 μg mL^{-1}, 3=20 μg mL^{-1}, 4=30 μg mL^{-1} and 40 μg mL^{-1})

Exposure of the Fenugreek seeds to the 20-nm-diameter AgNPs showed a clear and dose-dependent inhibitory effect on their subsequent germination success and on the growth of those seedlings that did germinate Fig. 5.

AgNPs at all concentrations affected the seedling growth, although this was not statistically significant at 10µg mL^{-1} for all parameters measured. The higher concentrations of AgNPs (10µg mL^{-1}) strongly inhibited both the shoot and root growth (especially as Moisture weight), with a more marked inhibition of the shoot growth than the root growth (Fig. 5).

Similar trends were also found in the root length (Fig. 5). The root length was reduced by 32 percent with 10µg mL^{-1} AgNPs (Table I), while the root dry weight was reduced by 29 percent, compared to the no AgNPs control.

TABLE I. EFFECT OF SILVER NANOPARTICLES ON SEED GERMINATION AND SEEDLING GROWTH OF FENUGREEK VALUES ARE AN AVERAGE OF THREE REPLICATIONS ±SE

No.	Concentration	% of Seed Germination	Speed of germination	Root length	Root fresh weight	Root Dry weight
1.	Control (0 µg mL^{-1})	64.44	3.26	52.50 A	2.096 A	0.931 A
2.	10 µg mL^{-1}	76.11	4.10	76.94	2.783	1.204
3.	20 µg mL^{-1}	75.74	4.07	76.38	2.766	1.192
4.	30 µg mL^{-1}	74.63	4.04	75.00	2.743	1.086 A
5.	40 µg mL^{-1}	70.74	3.96	69.44 A	2.587 A	1.035 A

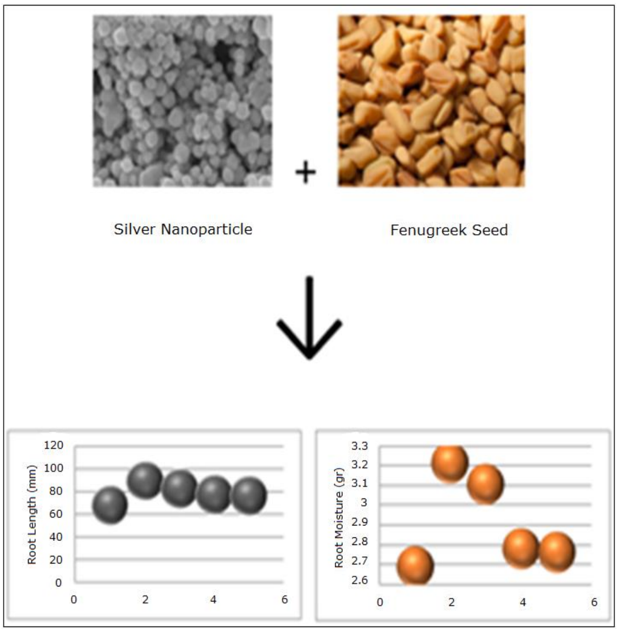

Figure 6. Graphical abstracts

IV. CONCLUSION

Nanomaterials are proposed to be the materials for the new millennium. Nanoparticles of size below 100nm fall in the transition zone between individual molecules and the corresponding bulk materials, which generate both positive and negative biological effects in living cell [18]. There is increasing amount of research on the biological effects of nanoparticles on higher plants. Only limited studies have been reported on the promotory effects of nanoparticles on plants in low concentrations.

Fenugreek responded to AgNPs with higher seeds germination percentage compared to control (Fig. 6). Graphical abstracts. The increased growth rate of the seedlings might be due to the enhanced uptake of water and nutrient by the treated seeds. These results suggest that release of AgNPs into the environment could have only positive effects on plant communities. Enhanced seed germination as well as early plant growth is vital to achieve crop productivity, especially for crops that otherwise show poor germination rates. The profound effect on the early stages of plant growth may be followed by similar enhancements at later stages as well, and by applying nanoparticles we may be able to improve plant productivity too.

In conclusion, these results of the current study reveal that the application of silver nanoparticles significantly enhanced seed germination potential. Application of silver nanoparticles improved percent seed germination, mean germination time, seed germination index, seed vigour index, seedling fresh weight and dry weight. It was found that the accumulation and uptake of nanoparticles was dependent on the exposure concentration.

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