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Effect of green synthesized silver nanoparticles on seed germination and seedling growth in wheat

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Abstract

Wheat is major food crop of world but unfortunately its production is decreasing day by day due to over-population therefore it is need of hour to execute modern technologies like nanotechnology to perk up the yield of important food crop. The green synthesis and application of metallic nanoparticles has become a significantly important branch of nanotechnology. In present study, we report the green synthesis of silver nanoparticles using leaf extract of *Moringa oliefera* as reducing agent and tested their effects on seed germination as well as seedling growth. The exposure of Silver to leaf extract resulted in rapid reduction of Silver ions leading to the formation of Silver NPs in the solution. Biologically synthesized Ag NPs were applied to evaluate the effect on seed germination and seedling growth of *Triticum aestivum* L. an economically important food crop. Seeds were treated with different concentrations of Ag NPs (25ppm, 50ppm, 75ppm and 100ppm) and tested against control. Application of all concentrations of Silver nanoparticles promoted seed germination and seedling growth in wheat as compared to control. However significant results were observed at 100ppm AgNPs as compared to control. Also, a significant increase in root fresh weight, root dry weight, root length, and root elongation was recorded at 100ppm concentration of Ag NPs as compared to the control.. The results of this experiment revealed that the application of Ag NPs enhanced the germination in wheat. So this study is quite helpful in exploring the potential of green synthesized silver nanoparticles to boost up the germination and seedling growth of wheat.

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Introduction

Triticum aestivum L. commonly called as wheat is one of the most important crop of family Poaceae. It occupies a position of supreme importance amongst all worlds' crop plants both in extent of area and magnitude of food production.

It occupies a large portion of cultivated area of globe and provides food security to all countries of world. So, it is second most significant consumables food crop in world following rice. Unfortunately yield of wheat crop has been decreasing from many years due changing climatic conditions. So, it is the need of the hour to increase the yield of major staple food of the world for the accomplishment of food requirements of the people by application of modern technologies (Asseng *et al*., 2011).

Nanotechnology is an innovative and promising field of interdisciplinary research. It founds a wide range of applications in various diverse fields like pharmaceutical, electronics, medicine, food processing, textile and agriculture. Nanotechnology has enormous potential uses as well as tremendous benefits.Basically nanoparticles are the atomic or molecular aggregates that possesses size dimension between 1 to 100nm. They have various unique physical as well as chemical properties in comparison to other bulk materials (Nel *et al.,* 2006).

Currently fabulous research has been carried out to investigate the positive effects of nanoparticles on the growth and development in various plants. However few studies also elucidated their negative impacts as well. Among diverse metallic nanoparticles, silver nanoparticles (AgNPs) are widely used owing to their unique physical and chemical properties like, catalysis, electrical conductivity, surface- enhanced Raman scattering (SERS) and magnetic and optical polarization. Besides, application of plant-based or green synthesized silver nanoparticles role in increasing plant growth and development (Ngo *et al*.,2014). Effectiveness of AgNPs is verified by their size, reactivity, composition, surface and most important one is the concentration at which they are effective (Khodakovskaya *et al.,*2012). Biologically synthesized silver nanoparticles promoted seed germination and seedling growth of *Boswellia ovalifoliolata* (Savithramma*e t al*., 2012). AgNPs application increased growth attributes such as root and shoot length and leaf area of *Zeyamays*, *Brassica juncea and Phaseolus vulgaris* (Salama2012; Sharma *et al.,* 2012).

Green synthesis is a cost effective, eco-friendly method to prepare nanoparticles which does not require high temperatures or pressures and importantly toxic chemicals.

It helps to have better progression than chemical and physical methods. Synthesizing nanoparticles using plants is more beneficent than any other biological ways as it would be difficult to maintain the cell cultures. Many other green methods were reported in the synthesis of nanoparticles using plant extracts (Mittal*et al*., 2013).

Seed germination is crucial stage in plant growth and it is also an important phenomenon in agriculture because it is regarded as thread of life of plants that ensure its survival (Zaka *et al.,* 2016).

The recent advances in nanotechnology and its applications and utilization in the field of agriculture is tremendously increasing; so it is quite persuading to study or explore the role of AgNPs in the germination of seeds.

Keeping in view the available literature, present study was planned to evaluate the effects of AgNPs on various aspects of wheat seed germination.

Materials and methods

The study was conducted following a complete randomized design with three replicates in the lab, Department of Botany PirMehr Ali Shah Arid Agriculture University Rawalpindi.

Green Synthesis of Silver nanoparticles

Green synthesis of Silver nanoparticles was carried out by using leaf extract of *Moringa oliefera* Plant.

Preparation of plant extract

For preparation of plant extract healthy and fresh green leaves of medicinal plant were collected and washed first with tap water followed by distilled water to eliminate all the dust and visible dirt particles.

After washing leaves were cut into tiny pieces and dried at room temperature. 10-20g of finely incised leaves was taken and transferred into 250 ml flask containing 100 ml distilled water and boiled for 15 minutes.

The extract was then filtered thrice by using Whatman No. 1 filter to remove all solid particles. The obtained clear solution was refrigerated at 4°C in 250 mL flask for further experiment. Sterility conditions were maintained at each and every step of the experiment to avoid contamination. (Banerjee *et al.*, 2014).

Method of preparing nanoparticles

Silver nano particles were synthesized by reducing silver nitrate ($AgNO_3$) with plant extract by modifying the method of Banerjee et *al.*, 2014. A solution of $AgNO_3$will be prepared by dissolving 238.31 mg of silver nitrate per litre of distill water.

This solution will be boiled and then reduced stepwise by addition of 10-20 ml of plant extract along with continuous boiling until color of solution changes to brown. Through this method stock solution of AgNPs 150 ppm concentration was obtained (Fig. 1). This stock solution was diluted for further treatments. UV visible spectroscopy and X-ray Diffraction analysis confirmed the synthesis of Silver nanoparticles (Fig.2a, Fig.2b).

Seeds collection

Seeds of wheat variety Glaxy13 used for the study were collected from National Agriculture Research Centre (NARC) Islamabad.

Experiment

Silver nanoparticles in different concentration (0, 25, 50, 75 and 100ppm) were prepared directly in distilled water and then dispersed by ultrasonic vibration for approximate one hour.All the seeds were stored in dark at room temperature. Viability of seeds was checked by suspending them in distilled water. The settled seeds at the bottom were selected for further experiment. The sterilization of seeds was carried out by suspending seeds in 5% sodium hypochlorite solution for 10 minutes and then rinsed thoroughly with distilled water.

After surface sterilization, the rinsed seeds stirred for about 2 hours in AgNPs suspension by using magnetic stirrer.

Three layers of filter paper were placed into each Petri plate and 5ml of the respective particle suspensions were added using a Pasteur pipette. Twenty five seeds were then transferred into each Petri dish at controlled temperature of 25±1°C.

Approximately 15ml of each concentration (0, 25, 50, 75 and 100ppm) of AgNPs was supplied to every test plantlets for 14 days. Distilled water was served as control. After 14 days of growth, the shoot and root lengths were long enough to be measured by using a scale.

A set of seeds was used without providing any treatment as a control. Germination rate of the seeds was recorded daily for fourteen days and Germination percentage was calculated till the last day.

The data for all the other growth related parameters which appeared early such as shoot length; root fresh weight and root biomass was recorded.

Germination percentage (GP)

Seeds were taken as germinated when radicle has emerged from the seed coat. Percentage germination frequency Percentage germination was recorded by using following formula. (Iqbal *et al.* 2016).

Germination Percentage = Number of seeds germinated /Total number of seeds ×100

Germination speed index (GSI)

The germination test was performed with daily calculation of that number of seeds that showed protrusion of primary root with length of ≥2mm always at the same time during entire trial. The germination speed index was calculated by sum of seeds germinated each day, divided by number of days passed between the seedling and germination [17]. According to Maguire formula

GSI= G1/n1+G2/n2+…….+Gi/ni

Where:

G= Number of seeds germinated each day

N= Number of days elapsed from seedling until the last count.

Seedling vigor index (SVI)

Seedling vigor Index was calculated by using method of Abdul-Baki and Anderson (1973) and expressed by Ushahra and Malik (2013).

Seedling Vigor Index = (Root length + Shoot length) × Germination Percentage.

Fresh and dry weights (g)

The fresh weight was estimated through weighing plant material in precision scale, and dry weight was calculated through weighing in precision scale after drying the material in oven at temperature of 70°C till constant weight. At the end of experiment, plumule and radical length as well as fresh weight was recorded. So dry weights were calculated by placing the plants in oven at 70°C for 48 hours and then weighed with sensitive scale.

Statistical analysis of data

Statistical analysis of each treatment was carried out with three replicates and all the results. Analysis of results was performed by one way Analysis of Variance by using Satatistix 8.1.

Results and discussion

In present study, different concentrations 0, 25, 50, 75 and 100ppm of AgNPs are prepared in distilled water and further used for the treatment in wheat seeds to explore their effects on seed germination and early seedling growth.Significant positive results on shoot length, root biomass and root length were observed for all seeds as compared untreated control germination.

Seed germination results revealed that AgNPs at all concentration enhanced seed germination and promoted seedling growth in wheat as compared to control plants.

Table 1. Effect of green Silver nanoparticles on seed germination and seedling growth of wheat (Mean values of three replicates).

Concentration of AgNPs	% Seed germination	Speed of germination	Seedling vigor index	Root fresh weight	Root biomass
Control (0ppm)	65.5 d	3.1 c	172 e	2.12 d	0.83 c
25ppm	70.2 c	3.8 b	193 d	2.31 c	1.01 bc
50ppm	74.6 b	4.4 ab	232 c	2.47 b	1.24 ab
75ppm	77.8 ab	4.6 ab	284 b	2.54 ab	1.36 ab
100ppm	82.3 a	5.1 a	372 a	2.92 a	1.49 a

The results revealed that the effect of AgNPs was significant on germination percentage. Highest germination percentage was recorded at 100ppm AgNPs.

Overall the results of this study showed that application of AgNPs can increase the germination percentage and seedling growth in wheat.

Among all the treatments 100ppm AgNPs proved best because highest values for germination percentage, germination time, seedling vigor index as well as seed germination index was recorded at this concentration (Fig. 3, 4, 5).

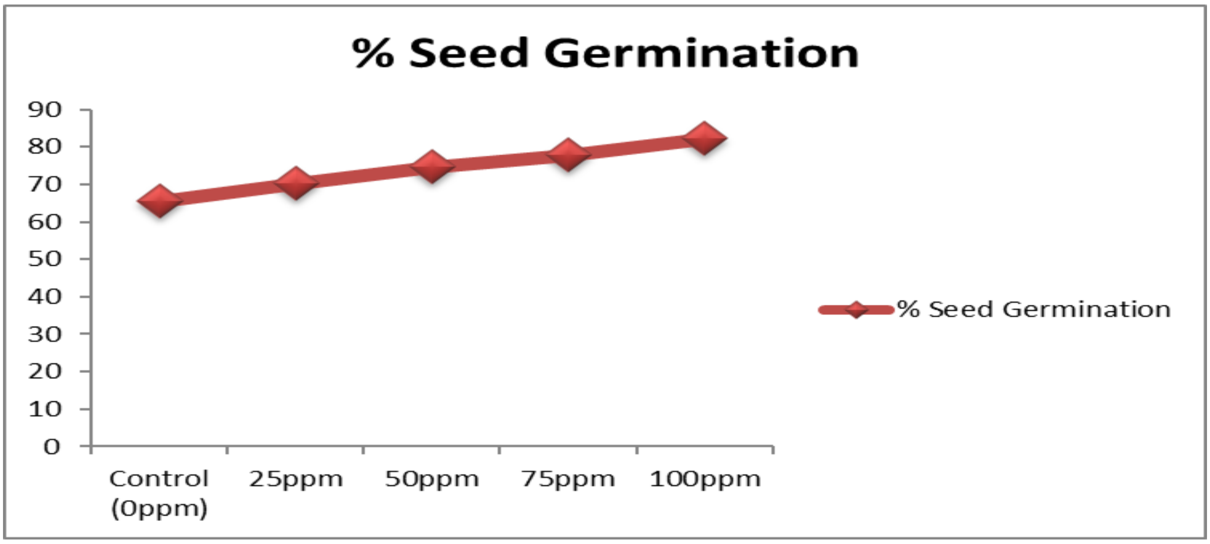

Fig. 1. Effect of AgNPs on germination percentage in wheat.

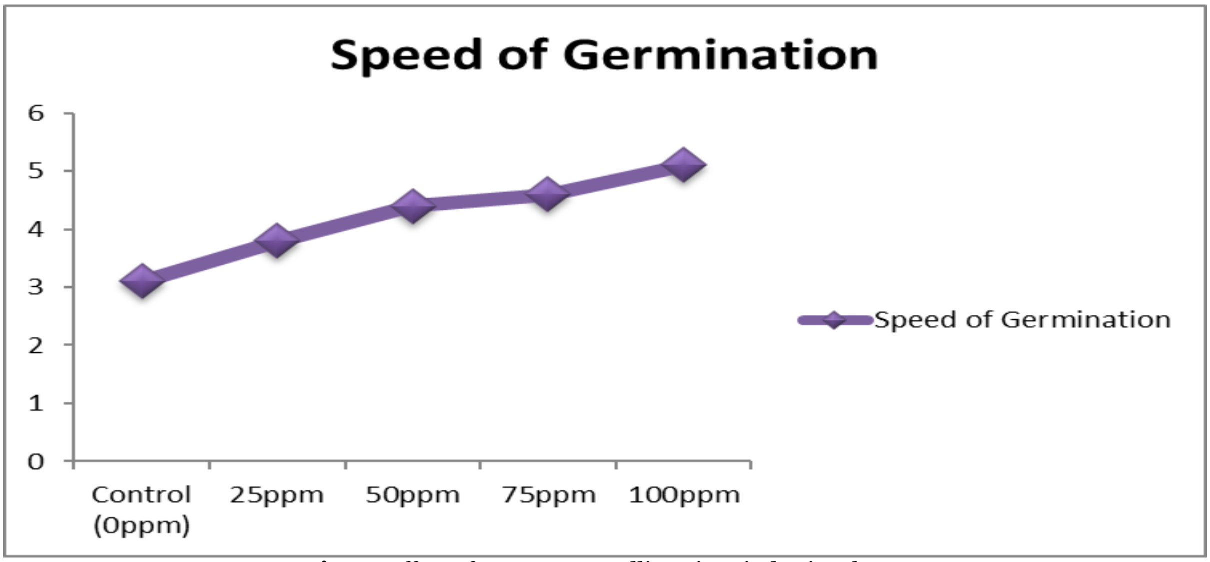

Fig. 2. Effect of AgNPs on seedling vigor index in wheat.

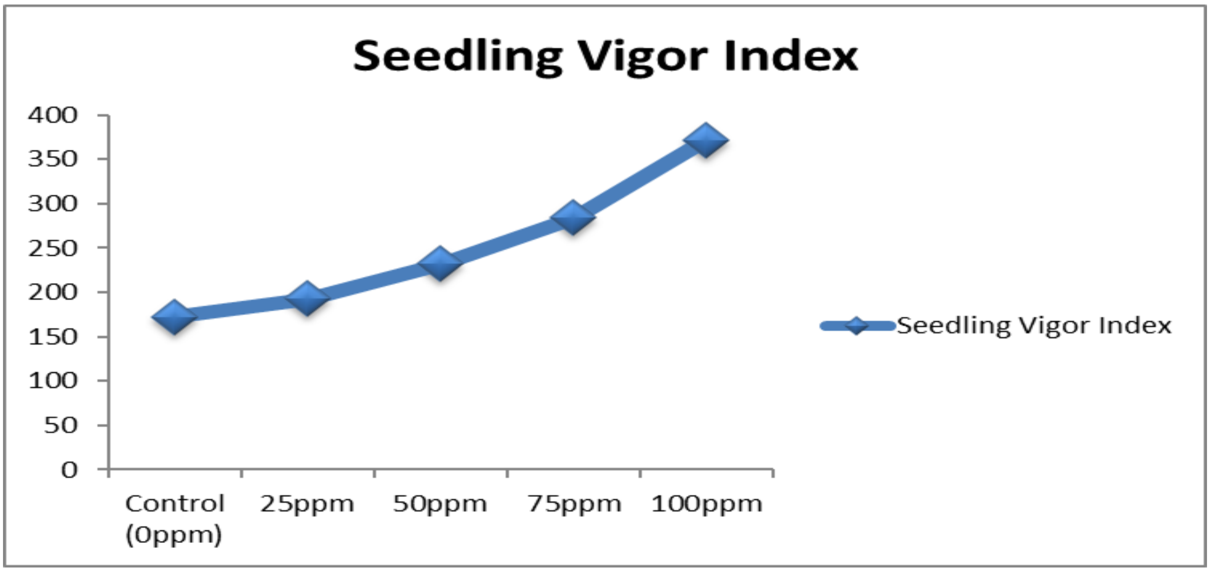

Fig. 3. Effect of AgNPs on seedling vigor index in wheat.

Root system was also well recognized and developed in less time in treated experiment as compared control. Highest values for root fresh weight and root biomass were also recorded at 100ppm AgNPs.

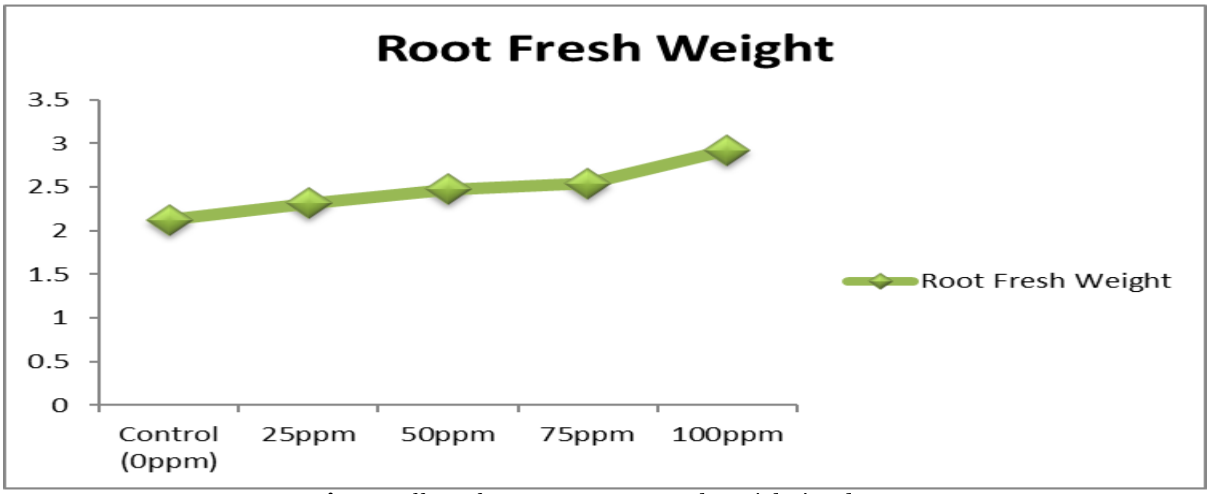

Fig. 4. Effect of AgNPs on Root Fresh Weight in wheat.

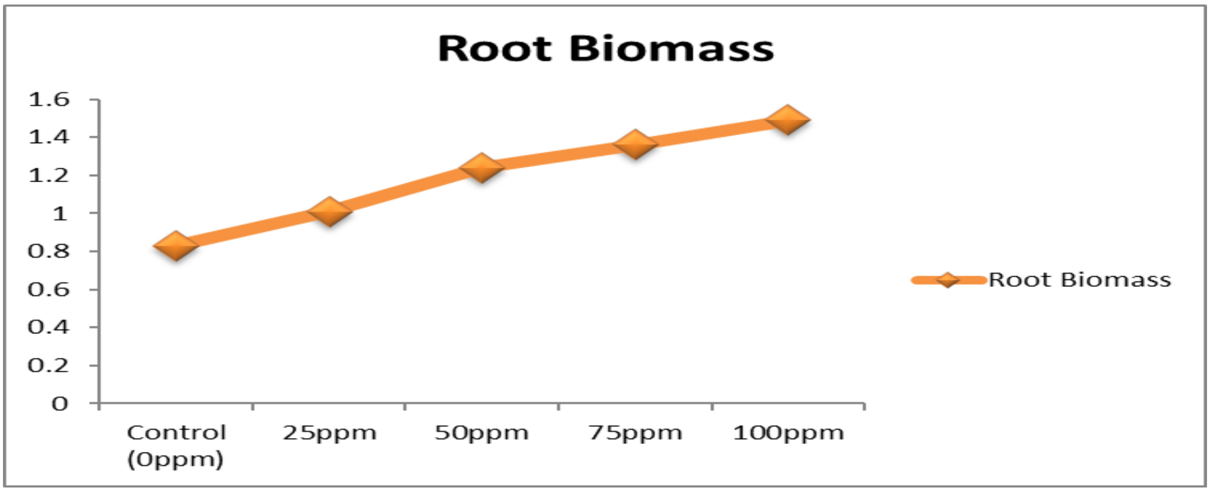

Fig. 5. Effect of AgNPs on Root Biomass in wheat.

So overall the results of present study showed that the level of seed germination and subsequent seedlings growth was increased with increasing concentration of AgNPs. Exposure of wheat seeds to AgNPs revealed tremendous effects on their consequent germination success and on the growth of those seedlings that did germinate.

Conclusion

Wheat seeds responded to AgNPs with higher seeds germination percentage compared to control. 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, the results of present study showed that the application of AgNPs significantly promoted seed germination potential in wheat. Application of AgNPs enhanced seed germination percentage, mean germination time, germination index, seedling vigor index as well as seedling fresh and dry weight. It was observed that the accumulation and uptake of silver nanoparticles was quite beneficial for wheat plants.

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