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Evaluation of phytotoxic impact of plant mediated silver nanoparticles on seed germination and growth of seedling of *Cassia occidentalis*

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Abstract
Background: The silver nanoparticles (AgNPs) are known for their antimicrobial activity against several pathogens. The AgNPs might entered into the environment that could adversely affect the herbs and plants.
Purpose: The purpose of the undertaken study was to synthesize AgNPs from *Vitex negundo* leaf extract and evaluate their effects on different growth parameters of *Cassia occidentalis*.
Methods: The AgNPs were synthesized by treating silver nitrate solution with aqueous solution of *Vitex nigundo* leaves extract. AgNPs were characterized by the analysis of UV-Visible absorptions spectra. The phytotoxic effects of these synthesized AgNPs on seed germination, shoot and root lengths, fresh weight and dry weight of seedlings and leaf surface area of *Cassia occidentalis* was evaluated in pre-sterilized petri dishes exposed to different concentration of AgNPs.
Results: The AgNPs from *Vitex nigundo* leaves extract were successfully synthesized and characterized. The results indicated that exposure of AgNPs causes both positive and negative effect on different growth parameters undertaken. AgNPs affected these parameters in concentration dependent manner. The AgNPs at different concentration of 20, 40, and 80 mg/L significantly increased the seed germination, shoot and root lengths, fresh weight and dry weight of seedlings, leaf surface area of *C. occidentalis*, While AgNPs at 100 mg/L and 1000 mg/L concentration significantly inhibit these parameters. The AgNPs (80 mg/L) was found to be optimal concentration that achieved the highest positive effects on the given parameters.
Conclusion: Finally, AgNPs exhibited both positive and negative effects on different growth parameters in concentration dependent manner. AgNPs can promote plant growth at their optimal concentration without causing any harm to the environment and plants.

Keywords: Phytotoxic, silver nanoparticles, seed germination, seedling growth, shoot length, root length, fresh weight, dry weight

Introduction
Nanotechnology is an important and leading multidisciplinary scientific field in material sciences, biotechnology, chemistry, pharmaceutical sciences and many other disciplines. Nonmaterial have very small particle size usually less than 100 nm. The unique size of nanoparticles plays essential role in many areas of scientific interest. Nanometalic particles (NPs) have cutting edge of the rapidly emergent field of nanotechnology. The NPs have large number of applications from industrial to household use. These NPs most likely entered to the environment, specially enter into the wastewater streams and finally included into sewage sludge and then finally entered to the fertile farming soil. Therefore, the risk assessment of these nanoparticles on the environment need a comprehensive study of their occurrence, mobility, eco-toxicity, and reactivity. Numerous published research articles reported the toxicity of NPs, especially cytotoxicity on mammalians, and other animals. However, limited research articles have been published pertaining to the toxicity of these nanoparticles on different flora. On the other hand, many research papers have been published that revealed the diverse and contradictory toxicity reports of these metallic nanoparticles in a number of plant species. The researchers around the globe are doing continuous efforts to find out and analyzed the toxicity effects of silver nanoparticles (AgNPs) against different plant species (Kaegi *et al*., 2010) [8]. A number of factors are involved in the mechanism and process of toxicity of the AgNPs against different plant species. The nanoparticle related factors involved in the toxicity of AgNPs are the size and concentration of AgNPs, while plant related factors involved in the toxicity of AgNPs are the age and species of the plant. The experimental conditions have also

Important in the toxicity of AgNPs which includes temperature, time of exposure, and And technique. This is reported that AgNPs inhibit the germination of the seed in *Hordeum vulgare* and decreased the length of the shoot in *Hordeum vulgare and Linum usitatissimum* at a specific concentration of 10 mg/L (EL- Temsah *et al*., 2012) [6]. In another study, AgNPs was found to inhibit germination of the seed, lipase enzyme activity and soluble as well as reducing sugar contents in *Brassica nigra* in a dose depending manner ranging from 0.2 to 1.6 mg/L. Contrary to this report, other study reported that AgNPs even at higher concentrations did not produced substantial effects on seed germination, length of the root, and shoot of the *Ricinus communis* L (Yasur and Rani, 2013) [22] and *Vicia faba* (Abdel- Azeem and Elsayed, 2013) [1]. Similar to these findings, the effect of AgNPs on seed germination in two different plants viz *Lactuca sativa* and *Cucumis sativus* was evaluated at a concentration of 100 mg/L and results of this study showed that AgNPs did not produced any significant effect on seed germination of these plants. While some other studies reported positive effects of AgNPs on seed germination. Sharma *et al*. (2012) [19] observed that AgNPs have some positive effects to enhance the growth of *Brassica juncea.* It has been concluded in some research studies that AgNPs positively affect seed germination in *Boswellia ovalifoliola* (Savithramma *et al*., 2012) [18], *Phytolacca Americana* and *Panicum virgatum* (Yin *et al*., 2012) [23], *Zea mays* and *Phaseolus vulgaris* (Salama, 2012) [17] and *Pennisetum glaucum* (Paraveen and Rao, 2014) [15]. It was observed that the plants exposed to some specific concentrations of AgNPs might lead to the improvement of the plant growth in comparison with control plants, It is documented that plant growth could affect negatively by lower and higher concentrations of AgNPs (Kaveh *et al*., 2014; Geisler *et al*., 2013; Qian *et al*., 2013) [10, 7, 16]. AgNPs positively affect a number of plant parameters e.g. root and shoot length, fresh weight, the vigour index of seedlings etc. Apart from the AgNPs, the other types of nanoparticles have inhibitory effects on plant growth at a higher concentration, while these nanoparticles at a low concentration augment the plant growth. It is also reported that multi-walled carbon nanotubes at low concentrations boost the growth of maize seedlings as compared to the exposure at higher concentrations (Tiwari *et al*., 2013) [20]. *Cassia occidentalis* L. (family: Leguminosae) is an important medicinal herb and It is widely used in Ayurveda as well as other traditional systems of medicine to prevent and cure of number of ailments. *C. occidentalis* have been reported to have antibacterial, anticancer, diuretics, anti-diabetic, antifungal, analgesic and anti-inflammatory, hepatoprotective, and laxative and potential. A number of phytochemicals were reported to be isolated from this unique herb. The examples some well-known chemical isolated from the *C. occidentalis* are aloe-emodin, emodin, anthraquinones, anthrones, apigenin, achrosin, aurantiobtusin, chrysophanic acid, campesterol, cassiollin, chryso-obtusin, chrysophanol, chrysarobin and chrysoeriol (Yadav *et al*., 2010) [21]. The seeds are easily germinated at ordinary atmospheric conditions. The plant "*Vitex negundo* L." (Family: Verbenaceae) is small tree having grey colored bark. This is very popular plant due to its well established pharmacological potentials against a large number of disorders. It is well accepted and widely used in different Indian traditional systems of medicine including Ayurveda. A numbers of pharmacologically active secondary metabolites e.g. alkaloids, flavonoids, glycosides, phenols, irridoid tannins and terpenes are present in all parts of this plant, mainly in its leaves. Innumerable therapeutic actions are attributed to the occurrence of these essential phytochemicals. This natural drug exhibited a variety of therapeutic actions such as antioxidant, anti-inflammatory, antimicrobial, astringent, anticancer. Bronchodilator, CNS-depressant, diuretic, detoxicant, and hepatoprotective activities. The leaf extract of *V. negundo* specifically reported to exhibit insecticidal, larvicidal, vermifuge, repellent, tranquilizer, nerve tonic activities (Basri *et al*., 2014) [5]. The purpose of the present investigation was to evaluate the phytotoxic effects of different concentrations of AgNPs derived from *Vitex nigundo* leaf extract on the germination of the seed and their impact on the growth of seedling of *Cassia occidentalis* in given standard conditions.

Materials and methods

Chemicals and reagents

Silver nitrate ($AgNO_3$, 99.5%) and PEG-400 from Merck were used in this study. The freshly prepared double distilled sterile water was used throughout of the experiment and for the preparation of the solutions. *Cassia occidentalis* seeds and *Vitex nigundo* leaf were collected from Herbal garden of Jamia Hamdard, India and authenticated by taxonomist. The voucher specimen (VN/FP/PP/25, CO/FP/PP/26) is deposited in the department. The seeds were spherical in shape and small in size. The seeds under this study were well preserved in a complete dried place while maintaining dark conditions.

Synthesis of Silver nanoparticle

All chemicals used in the experiments were analytical grade and was purchased from Merck Ltd. Nanoparticles were synthesized by biological method. Silver nitrate was reduced by aqueous solution of *Vitex negundo* leaf extract. The 1mM silver nitrate solution (90 ml) was transferred into a 250 ml capacity graduated glass beaker. Then, 10 ml of *V. negundo* leaf extract was added to this beaker and properly stirred this mixture. This mixture was then kept in a microwave oven (Samsung MW-73AD-B) working at 800W of power and 2450 MHz frequency. Subsequently, this solution was exposed to the microwave radiations for the duration of 90 seconds. The obtained supernatant was poured out and the nanoparticles were re-dispersed in distilled water. This process was further repeated three times to confirm the process of nanoparticles synthesis. The obtained pure sample was then freeze-dried in order to get dry AgNPs.

Preparation of nanoparticles suspensions

Silver nanoparticles stock suspensions were prepared by mixing pre-weighed silver nanoparticles (AgNPs) in double distilled water (DDW). The AgNPs were processed for ultra-sonication for the duration of 30 minutes to properly agitate the AgNPs suspension. The obtained AgNPs suspension was further stabilized by adding 10% (v/v) polyethylene glycol (PEG-400) according to the established method of Zhang *et al*. (2007) [7]. The final suspensions of AgNPs were again sonicated before the next use.

Surface sterilization and germination of seeds

The surface of the *C. occidentalis* seeds was sterilized by simply dipping the seeds into 0.1 % mercuric chloride solutions just for 10 minutes (Anonymous, 1996) [3]. The sterilized seeds were then properly washed and soaked with double distilled sterile water thrice. A piece of filter paper was placed on the sterile petridish, and AgNPs solution (5 ml) was carefully poured into it aseptically to avoid contamination. The pre-sterilized seeds of *C. occidentalis* were then kept on the surface of the filter paper in petridish. Each petridish having 10 sterilized seeds were incubated in an incubator to allow the germination (Kikui *et al*., 2005) [11]. One petridish without AgNPs solution (5 ml) was also undertaken that served as the control seeds group. The comparison of each test experiment was done with the control seeds group. The experiment was terminated after 12 days of initiation of the germination process. All parameters viz percentage (% age) of seed germinations, shoot length and root length of *C. occidentalis* seedlings in different treatment groups were precisely calculated.

Statistical analysis

Each treatment was replicated thrice and the results of the undertaken study were statistically calculated and tabulated as mean ± SEM (standard Error Mean). All experimental data generated in this study was compared with their corresponding negative control groups. The statistical analysis of the overall results was carried out by analysis of variance (ANOVA) using statistical package for social sciences (SPSS) Version 11.5.

Results and discussion

Synthesis of Silver nanoparticles

Nanoparticles were prepared by reduction of silver nitrate solution by *V. negundo* leaf extract. UV-Visual spectra of the newly synthesized AgNPs of *V. negundo* leaf extract shown absorbance peak at around 405 nm (figure- 1). The U.V-Vis absorptions spectra has been well established and sensitive evidence to prove the synthesis of colloidal silver nanoparticles. An intense absorptions peak was observed at 400 nm due to an intense surface plasmon resonance of the newly synthesized AgNPs. The symmetrical peaks indicates that there were no aggregations between the AgNPs.

Effect of Silver nanoparticles on seed germination

Nano biotechnology has been emerged as noble scientific field which become indispensable and integral part of different aspect of human life and material sciences. Many types of NPs have been shown to exhibit different toxic mechanisms in many test materials. Zinc nanoparticles have been known for its toxic effects in mice casing vomiting, diarrhea, and sometime may lead to death of mice. Likewise, Alumina and Aluminum nanoparticles have been known to have significant bioeffects in plant's growth. The results of our study demonstrated that higher concentration of AgNPs significantly inhibited seed germination rate, root and shoot growth. This outcome of the study indicates the complete conversions of silver ions into AgNPs. This is proved in our results that AgNPs exposure to the seeds significantly affects the seed germination, and seedling growth of the *Cassia occidentalis*. The optimum concentration of AgNPs was 80 mg/L that yielded the highest percentage of seed germination of *C. occidentalis* (figure-2). This improvement in the seed germination of *C. occidentalis* might be due to penetration of the nanoparticles into the seed coat which causes positive effects on the initiation and progression of seed germination. Further, it is also possible that nanoparticles might increase water absorption process by the germinating seeds (Zheng *et al*., 2005) [25], potentiate the seeds capabilities for absorption and consumption of more fertilizer and water, enhance the level of nitrate reductase enzyme, improve the antioxidant systems of the seed (Lu *et al*., 2002) [13] etc. On the other hand, nanoparticles might be responsible to reduce malonyldialdehyde level, superoxide radicals' contents and H_2O_2 level, while nanoparticles might cause improvement in the antioxidant enzyme system like ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase (Lei *et al*., 2008) [12]. To sum-up all these changes, nanoparticles might promote germination of the seeds in certain plant species. The variations in seed germinations, root growth and shoot growth were based on different concentrations of AgNPs. Overall, results evidently proved that higher concentrations of AgNPs have much adverse effect on seed germination and growth of various plant species.

Effect of silver nanoparticles on root and shoot length

The effect of AgNPs exposure on shoot length and root lengths of *C. occidentalis* are mentioned in Table 1. The shoot length and root length of the *C. occidentalis* increased in a concentration dependent manner of AgNPs. The shoot and root lengths were gradually increased as AgNPs concentration increased up to 80 mg/L. Interestingly, the shoot and root lengths were found to decrease at a concentration of 100 mg/L. It was noted that the growth of the shoot and root lengths were retarded at highest concentration of 1000 mg/L (Table-1). The retardation in the growth of the shoot and root lengths at higher doses indicated the toxicity of NPs at higher concentration. These findings were at par with Mahajan *et al*. (2011) [14].

Effect of Silver nanoparticles on fresh and dry weight

The effect of AgNPs on fresh weight and dry weight of *C. occidentalis* seedlings are in Table 2. The fresh weight and dry weight of *C. occidentalis* seedlings were found to be corresponding to their shoot length and root length as per given treatment of AgNPs. The fresh weight and dry weight of *C. occidentalis* seedlings were increased after treatment of AgNPs as compared with control group. Contrary to this, both fresh weight and dry weight of seedlings were recorded to be increased after treatment at the highest concentration (1000 mg/L) of AgNPs as compared with control group. The statistical analysis of the results showed that the fresh and dry weights of the seedlings were significantly ($P < 0.05$) greater than the untreated control group of plants at a concentration of 80 mg /L of AgNPs. These findings were at par with Zheng *et al*. (2005) [25].

Effect of Silver nanoparticles on leaf surface area

The results of the surface area of *C. occidentalis* leaf are presented in the Table 3. The surface area of the leaf was found to be significantly ($P<0.05$) increased in a concentration dependent manner upto definite concentration AgNPs (80 mg/L). The highest leaf surface area was achieved at a concentration of 80 mg/L as compared with normal control group. The leaf surface area was declined upon further increased in the AgNPs concentration till the highest concentration 1000 mg/L. These findings were at par with Karthick and Chitrakala (2011) [9].

Conclusion

The results of the present investigation indicated both positive and negative effect of AgNPs on different parameters undertaken including seed germination, shoot and root lengths, fresh weight and dry weight of seedlings, leaf surface area of *C. occidentalis*. AgNPs affected these parameters in concentration dependent manner. The AgNPs (80 mg/L) was found to be optimal concentration that achieved the highest seed germination, shoot and root lengths, fresh weight and dry weight of seedlings and leaf surface area of *C. occidentalis*. The mechanism of effects of AgNPs might be due to accumulation and uptake of these nanoparticles. Finally, it is concluded that plant growth can be promote by optimizing the AgNPs concentration without causing any harm to the environment and plant. Further studies are required to optimize the AgNPs concentration that can promote the growth of different plant species.

Conflict of interests

The authors confirmed that no conflicts of interest exists among them.

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Table 1: Effect of AgNPs on shoot length and root length of *C. occidentalis*

Treatment	Concentration (mg/L)	Shoot length (cm)	Root length (cm)
Control (DDW)	---	20.4 ± 3.4	9.8 ± 3.2
AgNPs	20	22.8 ± 2.8	13.8 ± 1.6
	40	28.8 ± 2.4*	15.5 ± 2.5*
	80	33.5 ± 3.6*	19.6 ± 1.7*
	100	22.7 ± 1.5	12 ± 2.9
	1000	15.6 ± 1.4	7.4 ± 2.4

Results are expressed as mean ± SEM. The mean value difference is significant with *P < 0.05. AgNPs = Silver nanoparticles, DDW = Double Distilled Water, SEM = Standard Error Mean, cm = centimeter, mg/L = milligram per liter

Table 2: Effect of AgNPs on fresh weight and dry weight of *C. occidentalis seedling*

Treatment	Concentration (mg/L	Fresh weight (gm)	Dry weight (gm)
Control (DDW)	---	9.5 ± 2.8	3.8 ± 1.6
AgNPs	20	11.7 ± 2.5	4.9 ± 1.5
	40	15.8 ± 1.4*	6.7 ± 1.8*
	80	20.6 ± 1.7*	8.4 ± 2.8*
	100	11..2 ± 2.4	4.1 ± 0.9
	1000	7.7 ± 1.3	2.2 ± 1.1

Results are expressed as mean ± SEM. The mean value difference is significant with *P < 0.05. AgNPs = Silver nanoparticles, DDW = Double Distilled Water, SEM = Standard Error Mean, cm = centimeter, mg/L = milligram per liter

Table 3: Effect of AgNPs on leaf surface area of *C. occidentalis*

Treatment	Concentration (mg/L	Leaf surface area (cm^2)
Control (DDW)	---	5.7 ± 1.2
AgNPs	20	6.1 ± 2.6
	40	7.2 ± 1.1*
	80	9.1 ± 2.8*
	100	5.1 ± 1.5
	1000	2.6 ± 0.8

Results are expressed as mean ± SEM. The mean value difference is significant with *P < 0.05. AgNPs = Silver nanoparticles, DDW = Double Distilled Water, SEM = Standard Error Mean, cm = centimeter, mg/L = milligram per liter

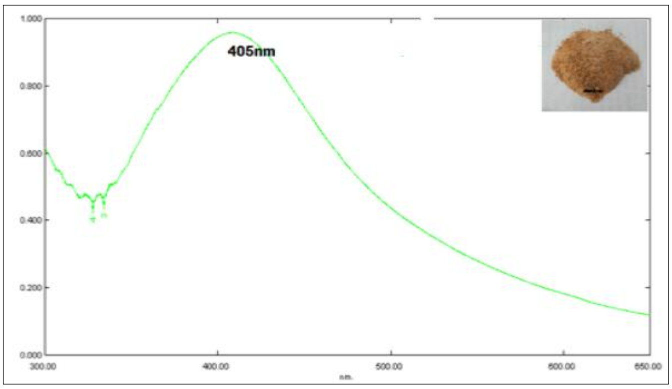

Fig 1: UV-Visible spectra of silver nanoparticles of *Vitex negundo*

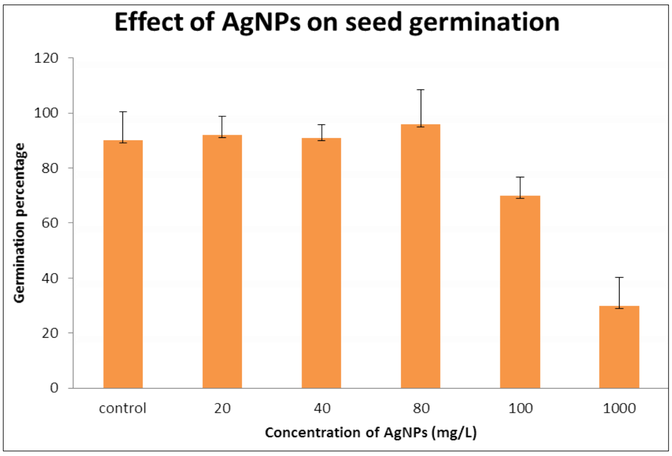

Fig 2: Effect of silver nanoparticles on seed germination of *C. occidentalis*

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