Effect Of Green Synthesised Silver Nanoparticles on Germination and Growth of *Allium parvum*

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Abstract: The advancement in nanotechnology has resulted in an exponential increase in the synthesis of nanoparticles like silver nanoparticles (AgNPs) and its wide spread use. This in turn, resulted in an increased leaching of these particles to the ecosystem, especially soil, from various industrial and household wastes. The alarming consequence of this cycle lies in the extent of toxicity that can be contributed by chemically synthesised NPs. In this study, we targeted a green synthesis protocol for AgNP preparation using the whole leaf extracts of *Aloe vera* L. and evaluated its effect on seed germination and growth of *Allium parvum.* The formation of AgNPs using *A. vera* whole leaf extract was confirmed by visual and UV- spectroscopic analysis. Our study showed that there is no significant variation in germination percentage between seeds grown in presence of water (control) and green synthesised AgNPs with 100% germination on day 3. Interestingly, the growth parameters indicated a slight decrease in the root length of green synthesised AgNP group on day 5, which can be attributed to the slight variation in initial germination time between the groups. Seeds grown in presence of the *A.vera* extract alone also showed similar germination and rooting pattern as that of control. Thus, the positive effects of green synthesised AgNPs on germination and growth might be partially due to the presence of the phyto-components in the plant extract. Even though phtotoxicity due to AgNPs are widely reported in many plant species, the production and use of these NPs cannot be replaced easily. In this context, our study opens the possibility for a safer option for AgNP preparation.

Keywords: green synthesis, silver nanoparticles, Allium parvum

INTRODUCTION

Silver nanoparticles (AgNPs) are nanosize (1-100 nm) particles of silver with unique physiochemical and biological properties which makes them suitable for various industrial applications with high commercial potential (Chaloupka *et al*., 2010). Since early 2000s, the applications of AgNP in food and agriculture industry have been reported; and hundreds of commercial agricultural products with AgNP have been marketed (He *et al*., 2019). It is estimated that by the coming years the AgNP application in food and beverage industry will increase many folds. Consequently, AgNP will be released into the environment and the majority will get accumulated in the terrestrial environment. This will pose critical adverse effects on the ecosystem and agriculture. The adverse effects of AgNP to crop plants were investigated by many researchers and possible mechanisms of adverse effects were reported as Ag intake, metabolic and proteomic response upon AgNP exposure etc (Mahakham *et al*., 2017; Anju *et al.,* 2022). This information provides a reference to design the sustainable strategies for synthesising metal nanoparticles for its use in industrial products in an environment friendly manner.

Metallic NPs are routinely synthesised by different chemical methods; which pose the serious issue of being toxic and less eco friendly (Popesco *et al*., 2010). There was an intense need for the development of cleaner and safer methods for NP synthesis. This pave way to green synthesis which utilizes microorganisms (Natarajan *et al*., 2010; Vigneshwaran *et al*., 2007; Kowshik *et al*., 2002) and plant extracts (Willner et al., 2006) for the reduction of silver to silver nanoparticles. The major disadvantage of the use of microbial source is the maintenance of aseptic conditions, high cost of isolation, and their maintenance in culture media due to which plants promise to be excellent sources for reducing agents for the synthesis of nanoparticles.

There are numerous examples of AgNPs synthesis from diverse plant sources, like (He *et al*., 2013; Sathishkumar *et al*., 2009). In the present study we targeted a plant of known antibacterial, antioxidant and medicinal properties for the green synthesis of AgNP. The present study used the leaf extract of *Aloe vera* as the reducing and stabilising agent for green synthesis of silver nanoparticles and evaluated the impact of AgNPs synthesised using green protocol on seed germination and plant growth capabilities of *Allium parvum.* The study will help in understanding the potential of green synthesised nanoparticles in sustainable agriculture practices.

MATERIALS AND METHODS

Preparation of *Aloe vera* Leaf Extract

The preparation of leaf extract was carried out as per the protocol of Khan *et al*., 2017 with slight modifications. Fresh *Aloe vera* leaves were collected and rinsed many times with distilled water to remove dirt particles and cut into small pieces. 20 g Aloe leaves was crumpled and shredded with a glass rod and after adding 200 ml sterilized distilled it is boiled for 20 min. After cooling the extract, it was filtered through Whatman filter paper and leaf extract was collected.

Preparation of Silver Nanoparticles

1 mM $AgNO_3$ solution was added to 20mL of the leaf extract drop by drop with continuous stirring. After that, 20 drops of 1% ammonia solution was added and shaken. The reduction of silver ions by the liquid plant extract, as well as the production of stable silver nanoparticles, resulted in a gray-black colour. The preparation procedure followed the earlier protocol of Khan *et al*., 2017 with slight modification.

Confirmation and Characterization of the Silver Nanoparticles

The confirmation of AgNPs formation by the reduction of Ag^+ to Ag^0 was confirmed by the colour change of solution from colourless to brown. Further the characterisation was done by reading the absorption peak using a UV–Vis spectrophotometer.

Measurement of Seed Germination and Root Growth.

Healthy seeds of *Allium parvum* were surface sterilized and was incubated in presence of water (control), chemically synthesised AgNP and green synthesised AgNPs on a rotary shaker 25 ± 1 °C for 1 h. The seeds were then transferred to sterile filters kept on petri dish (3 seeds per dish) and incubated under standard conditions of temperature and light for germination.

Seeds germinated showed a radicle extension of ≥ 3 mm and the final germination percentage (GP) was calculated as follows:

$$GP = Ng/Nt \times 100$$

where Ng is a total number of germinated seeds and Nt is a total number of seeds evaluated. The seedling germination capability was further examined by measuring the root growth. The spectrum was plotted in Origin software.

RESULTS AND DISCUSSION

Green Synthesis of AgNPs from *Aloe vera* Leaf Extract

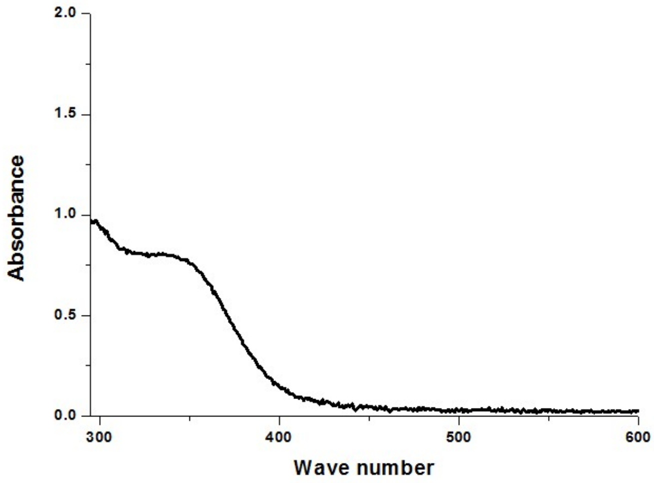
Green synthesis of silver nanoparticles was carried out in the present study using *Aloe vera* leaf extract. Bioreduction of silver ions into AgNP after addition of aqueous *Aloe vera* leaf extract was confirmed with change in colour. Initially, after addition of aqueous extract, the colour was pale yellow; with the increase in incubation time the colour changed from pale yellow to light brown. The setup was incubated in dark chamber to minimise photo-activation of silver nitrate at room temperature. The colour change from colourless to brown confirms the reduction of silver ions (Fig. 1). The colour change clearly indicates the formation of AgNP from the extract. Here, the phytocomponents present in the plant extract acts as the reductant which converts Ag^+ ions to metallic silver and crosslinks and stabilises Ag^0 to AgNPs.

Figure 1: Colour change of *Aloe vera* leaf extract during the synthesis of AgNP

***Aloe vera* leaf extract** | **Green synthesised AgNP**

The spectrum analysis of the AgNP formed using UV Visible spectrophotometer showed a strong excitation peak near 470nm (Fig. 2) corresponding to the surface Plasmon resonance of metallic silver. This confirms the formation of AgNP.

Figure 2: UV- Visible spectra of green synthesised AgNPs



Effect of AgNPs on Seed Germination Capability and Root Growth

The effect of chemically synthesised and green synthesized AgNPs on germination of *Allium parvum* seeds was monitored. Under standard conditions, control seeds showed 100% germination on the second day of incubation itself. The use of green synthesised AgNPs also showed 100% germination on day 3 (Table 1; Fig. 3A and 3B).

The influence of NPs on plant cells is intricate with varying effects. The same type of nanomaterial have shown different biological impacts (Hatami, 2017; Yan & Chen, 2019; Pacheco et al., 2017). Nanoparticles were reported to have both positive and negative impact on plant growth. One of the most studied methods of identifying the effect of nanoparticles on plant growth is the assessment of germination capability of seeds (Pacheco et al., 2017). In the present study, it was observed that the green synthesised AgNPs have no significant adverse effect on the germinating capability of an edible plant *Allium parvum*. The phytocomponents in plants like flavonoids and polyphenols are antioxidant molecules produced by plants and are involved in protection against stresses by mediating detoxification reactions, acting as metal chelators and participating in ROS scavenging through peroxidases (Thiruvengadam et al., 2015; Homaee & Ehsanpour, 2016). In green synthesised AgNPs, the silver ions are reduced and stabilised by phytocomponents in the leaf extract which might have conferred the positive impact of biologically synthesised AgNPs.

The root growth was analysed by measuring the length of root formation on day 5 of experiment. Interestingly, the growth parameters indicated a slight decrease in the root length of green synthesised AgNP group on day 5 (Table 1), which can be attributed to the slight variation in initial germination time between the groups. Seeds grown in presence of the *A.vera* extract alone also showed similar germination and rooting pattern as that of control. Thus, the positive effects of green synthesised AgNPs on germination and growth might be partially due to the presence of the phyto-components in the plant extract. Even though phtotoxicity due to AgNPs are widely reported in many plant species, the production and use of these NPs cannot be replaced easily. In this context, our study opens the possibility for a safer option for AgNP preparation.

Figure 3A: Germination percentage of experimental groups of *Allium parum*

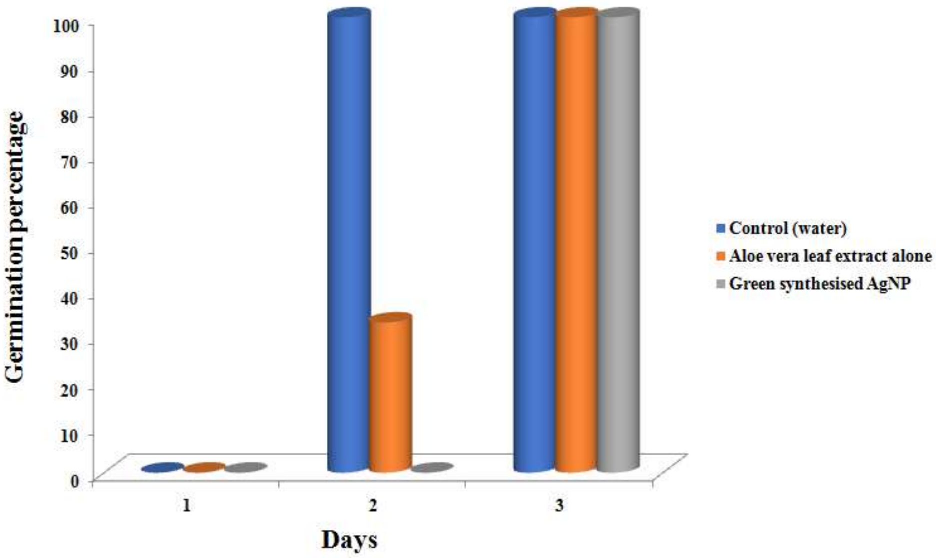


Figure 3B: Germination of *Allium Parvum* seeds on day 5 with control (water), *Aloe vera* leaf extract and green synthesised AgNP

Table 1: Germination percentage and root length of experimental groups of *Allium parvum*

Experimental group	Percentage of seeds germinated			Length of root on day 5 (cm)
	Day 1	Day 2	Day 3	
Control (water)	0	100	100	3.5
Aloe vera leaf extract alone	0	33	100	3.5
Green synthesised AgNP	0	0	100	0.9

CONCLUSION

In conclusion, a simple and safe process is utilized for the synthesis of silver nanoparticles by using the *Aloe vera* leaf extract extract. The extracts act as a reducing agent for nanoparticles synthesis. No chemical reagent or surfactant template is required in the process, which consequently established the bioprocess with the advantage of being environmentally friendly. The evaluation of the effect of green synthesised AgNPs on seed germination and root growth showed promising results for development of a suitable AgNP synthesis route without deleterious environmental hazards for future use.

ACKNOWLEDGEMENT

The authors acknowledge Newman College, Thodupuzha for providing the infrastructural facilities to conduct the work. This work was supported by the financial assistance from DBT, Govt. of India under DBT Star College Scheme to Newman College, Thodupuzha, Kerala

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