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Silver-chitosan nanoparticles induced biochemical variations of chickpea (*Cicer arietinum* L.)

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ABSTRACT

Chitosan-silver (Ag-CS) nanoparticles were synthesized and their physico-chemical characterization was carried out by DLS, TEM and FTIR. The study highlighted the reduced size (20–50 nm)and mono-disperse nature of Ag-CS Nps. Experiments were carried out to study the efficacy of Ag-CS Nps on chickpea seeds. Laboratory synthesized nanoparticles (0.1%, w/v) showed substantial growth promotory effect on chickpea seed germination, seedling length, fresh and dry weight. Regarding the pigment content, nanoparticles treated seedlings showed a remarkable increase of chlorophyll. A consequential increase in enzyme activity including α, β-amylase, ascorbate peroxidase (APX), peroxidase (POD) and catalase (CAT) was observed with nanoparticle treatment. In addition, Ag-CS Nps showed higher expression of MDA content. The overall results confirm the significant growth promotory effect as well as biochemical variation capabilities of Ag-CS Nps. This study opens up the possibility to use Ag-CS Nps as growth promotors in chickpea under the pot and field condition with the knowledge of toxicity levels.

1. Introduction

Any material when attenuated at the nanometer scale (less than 100 nm) exhibits new properties that are entirely different from its bulk counterpart due to small size and high surface to volume ratio. It is shown in terms of higher plasticity at high temperature and hardness, breaking strength, toughness at low temperature; higher chemical reactivity and surface energy; and high mobility in the body of an organism including cellular entry (Rajan, 2004). Nanotechnology plays an important role in enhancement of agricultural productivity using bio-conjugated nanoparticles. A variety of metal nanoparticles such as aluminum (Al), silica (Si), zinc (Zn) and metal oxide-based polymers including Zinc oxide (ZnO) and titanium dioxide (TiO_2) are being developed for agricultural uses (Barik et al., 2008; Nair et al., 2010; Khot et al., 2012). In the synthesis of metal nanoparticles the use of polymers like chitosan, soluble starch act as reducing agent in aqueous to control the particle growth, limit oxidation and stabilize the nanoparticle dispersion (Maciollek and Ritter, 2014). The synthesis of Ag-NPs using chitosan as both a reducing and capping agent has been reported by Sanpui et al. (2008).

Chitosan, a cationic polysaccharide with β-(1-4)-linked d-glucosamine (deacetylated unit) and N-acetyl-d-glucosamine (acetylated unit) has been considered as good material in agricultural nanotechnology owing to its biocompatibility, biodegradability, non-toxicity and antimicrobial property (Saharan et al., 2013). It is the second most plentiful natural biopolymer and is relatively cheap (Ma et al., 2008). The positive effect of nanoparticles in crop plants includes enhanced germination rate; root and shoot length, biomass of seedlings, photosynthetic activity and nitrogen metabolism (Zheng et al., 2005; Khodakovskaya et al., 2009; Anusuya and Sathiyabama, 2015). Lu et al. (2002) reported that application of nano SiO_2 and TiO_2 with soybean (*Glycine max*) plants accelerated the germination rate and growth. Most recently growth promotory effect of Cu-chitosan nanoparticles was reported that by Saharan et al. (2015).

Silver nanoparticles are of particular interest due to their role as substrates in the studies of catalysis (Tsujino and Matsumura, 2005; Shimizu et al., 2010). In the field of agriculture, the use of silver nanoparticles are relatively new and only very few literatures exist about the impact of silver nanoparticles on the physiology of crop plants. Therefore, an attempt has been made to elucidate the potential effects of Ag-chitosan (Ag-CS) NPs on growth and biochemical variations of chickpea under *in vitro* condition.

2. Materials and methods

2.1. Preparation and characterization of Ag-chitosan nanoparticles

Briefly, 100 ml of 0.1% (w/v) chitosan solution was prepared

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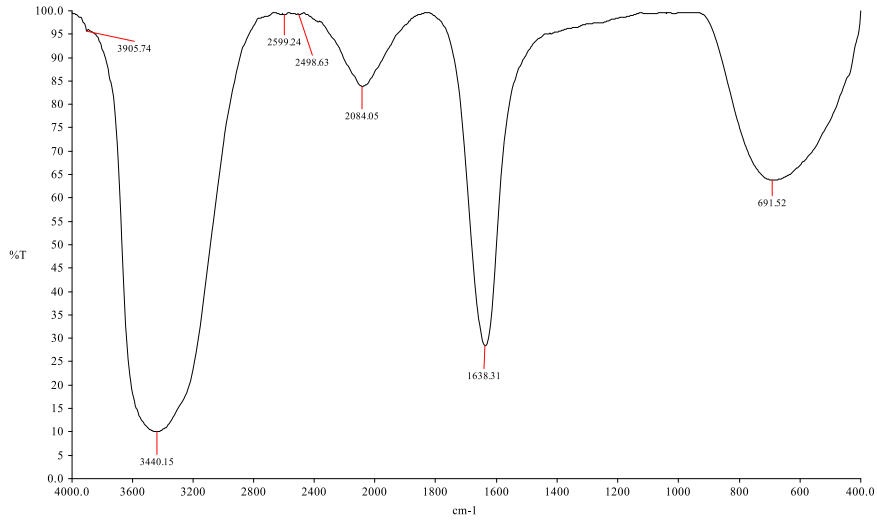

Fig. 1. FTIR spectrum of Ag-CS nanoparticle.

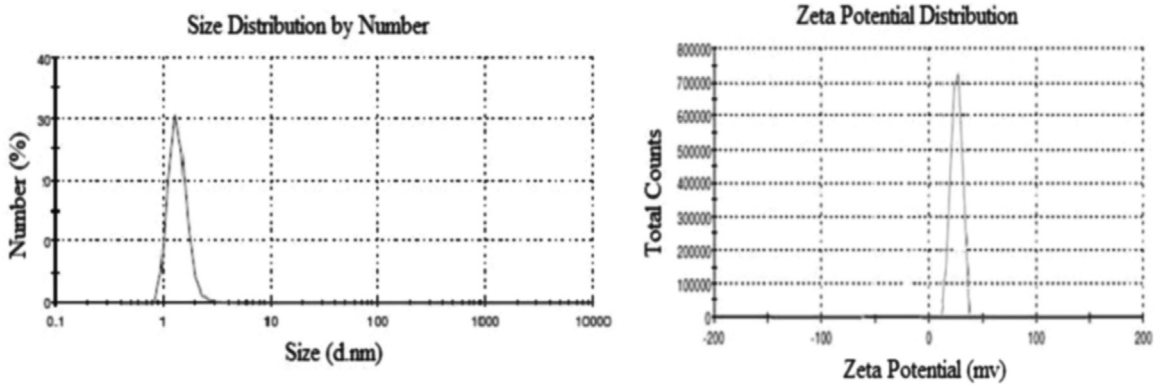

Fig. 2. Dynamic light scattering (a) and zeta potential of Ag-CS nanoparticle.

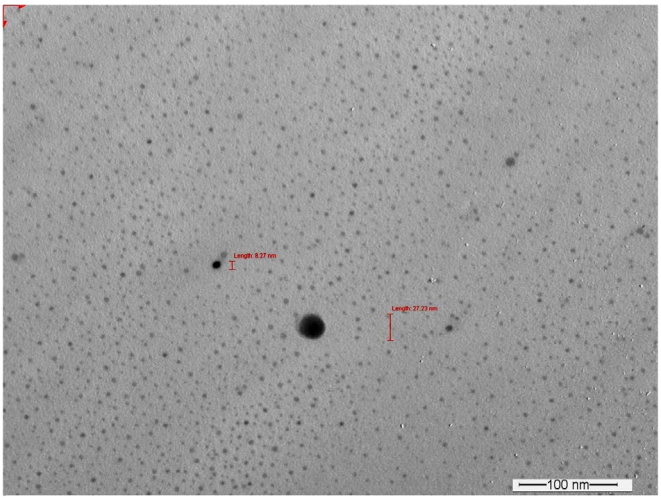

Fig. 3. HRTEM micrograph of Ag-CS nanoparticle.

using (0.1%, w/v) acetic acid, precipitated with 10N NaOH and centrifuged at 9000 g for 30 min. The supernatants were discarded and extensively rinsed with distilled water to remove any sodium hydroxide. Ag-CS NPs were prepared by adding 25 ml of 0.01% (w/v) silver nitrate solution to chitosan solution under magnetic stirring for 12 h at 70° C. It was then centrifuged at 10,000 g for 10 min, freeze dried and stored for further use.

The mean particle size, polydispersity index (PDI) and zeta potential of developed nanoparticles was performed by DLS on a Zetasizer (Malvern Instruments, UK) at 25 °C at a scattering angle of 90° in triplicate. FTIR analysis was done to confirm the functional groups of nanoparticles. The results were recorded by Nicolet 560 FT-IR spectrometer in a range of 400–4000 cm^{-1} using KBr pellet method. The size and morphology of the Ag-CS NPs were examined by HRTEM (JEOL model 1200 EX).

2.2. Seedling bioassay

Chickpea seeds (*Cicer arietinum* L.) obtained from local farm was used to determine the efficacy of Ag-chitosan nanoparticles on seedling growth using standard methods with some modifications (International Seed Testing Association, 1976). Briefly, chickpea seeds were surface sterilized by immersing in 0.01% sodium hypochlorite solution for 10 min and then rinsed thrice with deionized water. The sterilized seeds were placed in Petri plates having filter paper with 5 ml deionized water as control and 5 ml of Ag–chitosan NPs (0.1%, w/v) as treated. Each treatment was performed in triplicates with 10 seeds in each plate. Sealed Petri plates were maintained at 28 ± 2 °C in a growth chamber. Data were recorded for seed germination percentage, mean seedling length, fresh and dry weight at different intervals (2, 4, 6, 8 and 10 d). Seedlings which emerged out of soil level were considered for computation of germination percentage. Seedling vigor index (SVI) was calculated by the formula described by Abdul–Baki and Anderson (1973). Seed vigor index = (germination %) × (seedling length).