Acta Physiol Plant (2016) 38:58
DOI 10.1007/s11738-016-2074-1

ORIGINAL ARTICLE

# Effect of synthetic and biosynthesized silver nanoparticles on growth, physiology and oxidative stress of water hyacinth: *Eichhornia crassipes (Mart) Solms*

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Received: 20 June 2015 / Revised: 14 January 2016 / Accepted: 18 January 2016 / Published online: 1 February 2016


**Abstract** Silver (Ag) nanoparticles (NPs) are synthesized by several methods and are being widely used in various fields of science. In recent times, evaluation of their toxicological effects on environment, especially to the plant ecosystems has attained special attention. In this study, effect of synthesized AgNPs [chemically (S-AgNPs) and/or biologically (B-AgNPs)] on the growth and physiology of an aquatic plant water hyacinth—*Eichhornia crassipes* (Mart) Solms was evaluated. Water hyacinth plants were treated with S-AgNPs and B-AgNPs at different concentrations of 1, 10 and 100 mg $L^{-1}$and growth was monitored for 5 days. Decreased growth of hyacinth was observed only on fifth day in treatments with S-AgNPs treatment alone but not for B-AgNPs. Further, the atomic absorption spectroscopy results (at 100 mg $L^{-1}$ concentration) showed a higher accumulation of S-AgNPs over the B-AgNPs in various parts of the treated plants. Biochemical analysis on day five in B-AgNPs treated leaf extracts revealed an increase in carbohydrate and protein levels, and a decrease in phenol and chlorophyll content. In contrary, S-AgNPs treated leaf extracts did not show any significant changes in carbohydrate and protein levels, however, observed a significant increase in phenol and chlorophyll content. Interestingly, S-AgNP treatment increased the activities of antioxidative enzymes, such as catalase, peroxidase and superoxide dismutase. No significant differences were measured in plants treated with B-AgNPs when compared to normal plants which may reveal that these B-AgNPs instead enhanced the plant growth with a fewer minor effects on water hyacinth plants over S-AgNPs.

**Keywords** Silver nanoparticles · Water hyacinth · Antioxidative enzymes · Biochemical parameters · Biosynthesized nanoparticles

**Abbreviations**

| | |
|---|---|
| ROS | Reactive oxygen species |
| AgNPs | Silver nanoparticles |
| $AgNO_3$ | Silver nitrate |
| POD | Peroxidase |
| SOD | Superoxide dismutase |
| CHL | Chlorophyll |
| CAT | Catalase |

## Introduction

Nanomaterials are extensively used in almost every field of science, owing to their small size and large surface area. Silver nanoparticles (AgNPs) are reported to have antimicrobial properties (Luoma 2008; Tolaymat et al. 2010; Fabrega et al. 2011; Chernousova and Epple 2013) and are being widely used in several products that find applications in day to day life such as washing machines, socks, medical bandages, water purifiers, etc. (Ratte 1999; Silver 2003; Nowack et al. 2011). Due to their wide usage, these NPs leach into the aquatic environment (Benn and Westerhoff 2008; Fabrega et al. 2011; Farkas et al. 2011), through household or industrial effluents, and influence the growth

Communicated by A Krolicka.

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and metabolism of aquatic organisms (Griffitt et al. 2008; Gaiser et al. 2011) and plants. Aquatic plants, *Eichhornia crassipes* and *Pistia stratiotes* are used for the phytoremediation of waters contaminated with low levels of heavy metals (Qian et al. 1999; Odjegba and Fasidi 2007). However, this property of metal accumulation affects the growth of plants (Nagajyoti et al. 2010). Similarly, exposure to NPs have shown several effects on seed germination, root and shoot growth in various plants due to translocation of carbon nanotubes in rice plants (Lin et al. 2009), copper nanoparticles in wheat and mung bean (Lee et al. 2008), and many other plants (Lee et al. 2010; Ma et al. 2010). Recently, awareness has been drawn towards the toxic effects of AgNPs on aquatic plants. It has been reported that AgNPs hamper the growth of *Lemna minor* (Gubbins et al. 2011) and *Lemna gibba* (Oukarroum et al. 2013). Various other studies have shown that AgNPs application had toxic effects on 11 species of common wetland plants (Yin et al. 2012); At similar concentrations used, 6-nm gum arabic coated—AgNPs (GA–AgNPs) showed pronounced toxic effects on *Lolium multiflorum* than $Ag^{+}$ ($AgNO_3$) (Yin et al. 2011). Lately AgNP suspension exposure, had inhibited the growth of *Lemna paucicostata* (Kim et al. 2011), leading a path to study the effects of AgNP suspension on aquatic plants.

It is prudent to ascertain that the toxicity of nanomaterial is based on several parameters such as size, shape, charge and type of synthesis (Pal et al. 2007; Miralles et al. 2012; Thwala et al. 2013). As indicated in earlier studies, NPs have both positive and negative effects on various biological functions of plants (Navarro et al. 2008; Rico et al. 2011). It is apparent from our previous work the biogenic AgNPs have comparatively less toxic effects on *Daphnia magna* than chemically synthesized AgNPs (Usha Rani and Rajasekharreddy 2011). In recent times, biosynthesized AgNPs are increasingly used in various applications, despite the limited availability of data on their toxicological effects, especially on ecosystems and environment. But the chemical properties of silver ions facilitate their uptake through cell membrane and lead to accumulation by the organisms (Luoma 2008). In the current study, the effects of S-AgNPs and B-AgNPs that are synthesised using different methods and possess different coating properties were evaluated on the growth and physiology of water hyacinth plants.

Similar to other metals, AgNPs induce the oxidative stress by formation of reactive oxygen species (ROS) (Mittler 2002; Nel et al. 2006) and are scavenged by various anti-oxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Hegedus et al. 2001) in plants. Recent studies indicated that oxidative damage is one of the processes involved in the toxicity of AgNPs to animals, bacteria, and algae. In our previous findings, application of S-AgNPs on castor bean seeds though did not negatively affect their germination, but induced changes in antioxidative enzymes such as CAT, POD and SOD (Jyothsna and Usha Rani 2013). It has already been stated in other works that AgNPs can cause oxidative stress in *L. gibba* (Oukarroum et al. 2013); however, there are no efficient studies on the responses of antioxidant system of the aquatic plants to AgNPs treatment. In addition, the impact of S-AgNPs and B-AgNPs on the aquatic plants remains unexplored and hence we worked on the effects of these synthetic and biosynthesized AgNPs on aquatic plant, water hyacinth.

Water hyacinth (*E. crassipes*) is an aquatic weed and has received significant attention by researchers for its possible biosorbent property; its ability to grow and remove the toxic heavy metals from the polluted water (Mahamadi and Nharingo 2010a, b), and as an agent of phytoremediation (Malik 2007). Further, it has also been reported by Mahmood et al. (2010) that it can act as a hyperaccumulator, while observing the ashes of water hyacinth with $Pb^{2+}$, $Cr^{6+,}$ $Zn^{2+}$ and $Ni^{2+}$. However, the data on impact of the synthesized AgNPs on aquatic plants is currently limited. Thus, a detailed study was undertaken to explore the effects of synthesized AgNPs on biochemical and antioxidative enzymes of water hyacinth plant.

The current study was designed to assess the impact of bioaccumulation of AgNPs in water hyacinth plants, through evaluation of critical parameters such as plant growth, pigment content and activity of antioxidant enzymes. Considering the increased use of biosynthesized nanoparticles, we conducted the experiments using biologically synthesized AgNPs and compared with chemically synthesized NPs showing the differences that might arise due to different production processes.

## Materials and methods

### Plant material and growth conditions

Water hyacinth—*E. crassipes* plants were collected from the ponds near Hyderabad. Plants with longer petioles were selected for the studies and segregated according to their size and shape for uniformity. Plants showing any evidence of damaged tissue or leaf were excluded from the experiments. Epiphytes, insect larvae and sediments were removed by several washings with tap water and acclimatized to laboratory conditions in a fresh tap water for about a week. Plants were maintained in a growth chamber at 25 ± 2 °C on a 16 h light/8 h dark cycle.

### Preparation of silver suspensions

Powdered AgNPs that are stabilised with polyvinyl pyrrolidone (PVP) of <100 nm size was purchased from

Sigma Aldrich Inc., USA. These nanoparticles are termed as chemically synthesized silver nanoparticles S-AgNPs, and were dispersed in the water (Millipore water) and sonicated to prevent aggregation. Size of these AgNPs was found to be 70 nm as reported earlier (Jyothsna and Usha Rani 2013), and the hydrodynamic size and zeta potential of the AgNPs were 219.5 nm (*Z* average value of 167.9 nm) and $-22.3 \pm 5.78$ mV, respectively.

Biosynthesized AgNPs were produced using leaves of *Ricinus communis* L. The plant leaf broth solution was prepared following the method of Song and Kim (2008), with slight modifications. The synthesis was carried out by taking 10 mL of leaf broth, addition of 190 mL of $1 \times 10^{-3}$ M aqueous $AgNO_3$ and irradiated under direct sunlight exposure with a clear sky conditions (Rajasekharreddy et al. 2010). The resulting dry powder, biosynthesized AgNP (B-AgNPs) was used in the experiments. Particle size was around $20 \pm 7$ nm (H-7500 Transmission Electron Microscope, Hitachi, Japan) (Fig. 1) and the zeta potential was $-34 \pm 7.78$ mV (measured using Zetasizer Nano Series, Malvern Instruments, UK).

### Treatment with nanoparticles

Stock solution (100 mg $L^{-1}$) of AgNPs was serially diluted in Millipore water to obtain different concentrations—1, 10 and 100 mg $L^{-1}$. Healthy water hyacinth plants were transferred to 250 mL beakers containing 100 mL tap water and different AgNP concs prepared were added to these glass beakers to achieve the final concentrations—1, 10 and 100 mg $L^{-1}$. Tap water without any treatment served as controls. Water loss due to transpiration and evaporation processes is compensated daily by addition of fresh tap water. Five replicates were maintained for each concentration. Laboratory conditions were maintained at $28 \pm 2$ °C and $65 \pm 5$ % of humidity for all experiments. Physical parameters such as leaf width, leaf height, stem length, bud diameter, root length and plant height were measured before and after the treatments. As the changes in the plant height were prominent, they were considered to describe the growth in water hyacinth plants on treatment. All the parameters were noted on 1, 3 and 5 days. But the data recorded on the fifth day was considered for statistical analysis. The leaf materials were collected on fifth day of treatment for biochemical analysis to determine carbohydrate, protein, phenol, chlorophyll, metal content and for the measurement of antioxidative enzyme activities.

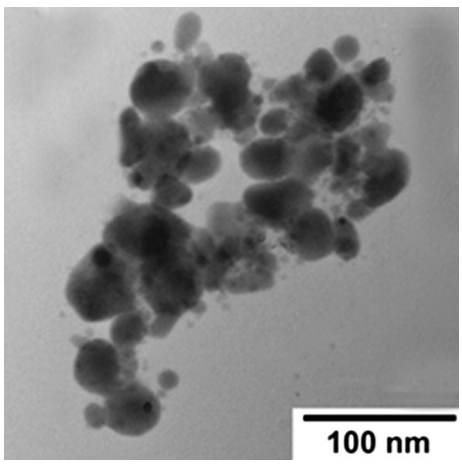


**Fig. 1** Transmission electron microscopic image of the biosynthesised silver nanoparticles

### Absorption and accumulation of silver nanoparticles

Uptake of AgNPs by water hyacinth plants was determined by quantifying the amount of silver in the treated and untreated plant tissue. Acid hydrolysis was carried out after drying the plant material at 90 °C in a hot air oven for 48 h (until they became ash black). Digestion of samples was performed using an acidic mixture of 40:4:1 (nitric acid: perchloric acid: sulphuric acid). For acid digestion of plant material, 1 g of sample was placed in an erlenmeyer flask and 10 mL of acid mixture was added. The plant tissue was digested by heating up to 150 °C for 4 h to obtain a colourless solution and filtered through Whatman No. 1 filter paper. Distilled water was added to the filtrate to achieve a final volume of 25 mL. Silver concentration in the samples was quantified using Flame Atomic Absorption Spectrophotometer (Perkin Elmer, AAnalyst 300).

### Estimation of carbohydrate and protein contents

Biochemical contents carbohydrates and proteins, in the water hyacinth leaves were estimated following the Dubois method (Dubois et al. 1956) and Lowry method (Lowry et al. 1951). Quantity of the contents estimated was expressed as microgram per gram fresh weight (μg/g FW).

### Estimation of total phenol and chlorophyll content

Phenol estimation was carried out by homogenizing 1 g of leaf material with 10 mL of 80 % methanol and agitated at 70 °C for 15 min. Extract thus obtained was filtered and stored at −80 °C until further use. Leaf extracts (100 μL) of different concentrations were diluted with 5 mL of distilled water followed by the addition of 250 μL Folin's reagent and incubated at room temperature for 3 min. Thereafter, 1 mL of 20 % $Na_2CO_3$ solution and distilled water were added and incubated for another 1 h at 25 °C. Finally the absorbance was read at 725 nm.

Chlorophyll content (CT) was analysed by homogenizing the leaf material (0.25 g) with 5 mL of 80 % acetone and incubated at 4 °C in dark until leaves became colourless (Lichtenthaler 1987). The chlorophyll A and B contents were measured by reading the absorbance, respectively at 645 and 663 nm using UV–Vis Spectrophotometer (Spectra Max M3, Molecular devices). The chlorophyll content of the replicates of different concentrations were calculated using the formula,

$$CT = 20.2 \times A_{645\,nm} + 8.02 \times A_{663\,nm}$$

where, CT = chlorophyll content, $A_{645\ nm}$ = absorbance at 645 nm, $A_{663\ nm}$ = absorbance at 663 nm.

### Assessment of antioxidant enzyme activity

Crude extract for the enzyme assays was prepared following the method described by De Biasi et al. (2003) with slight modifications. Briefly, 0.2 g of leaf was ground in 5 mL 0.1 M potassium phosphate buffer (pH 7.0) in a ceramic mortar and pestle. The samples were centrifuged at 6000×*g* for 15 min and the supernatant collected was used for the estimations.

Catalase activity was measured according to the Aebi (1984) with slight changes. Reaction mixture contained 2.8 mL of 50 mM phosphate buffer (pH 7.0), 80 µL of 0.05 M $H_2O_2$ and 120 µL of the crude extract. Absorbances of the samples were read at 240 nm using spectrophotometer. One unit of the enzyme was defined as 1 mol of $H_2O_2$ decomposed per minute and the activity denoted as unit per gram fresh leaf weight.

Peroxidase activity was determined according to the method of Chance and Maehly (1955) with slight modifications. Reaction mixture contained 10 µL of the leaf extract, 250 µL of 1 % $H_2O_2$, 500 µL pyrogallol and 990 µL of 0.1 M potassium phosphate buffer (pH −7). The change in absorbance at 420 nm due to the oxidation of pyrogallol in the presence of $H_2O_2$ was measured and kinetic readings were taken for 3 min at an interval of 30 s. One Peroxidase unit was described as the change of 1.0 absorbance unit per mL enzyme extract per min and expressed as unit of enzyme activity per gram fresh weight of the leaf material.

Superoxide dismutase activity was measured according to Beyer and Fridovich (1987) method. 30 mL reaction mixture contained 27 mL of 50 mM potassium phosphate buffer (pH 7.8), 1.5 mL of 10 mM methionine, 1 mL of 57 µM nitroblue tetrazolium (NBT) and 0.75 mL 0.025 % (v/v) Triton X-100. To 3 mL reaction mixture, 60 µL of crude leaf extracts and 30 µL of 1 µM riboflavin were added to test tubes. The mixture was immediately vortexed and illuminated for 15 min under fluorescent lamps. Later the change in absorbance was recorded at 560 nm. SOD activity was described as amount of enzyme required to produce 50 % inhibition of the NBT photoreduction.

### Statistical analysis

The results were presented as mean of five replicates with standard error (SE) and the values are compared between treatment and concurrent controls. Data were analysed by using analysis of variance (ANOVA), and the means were statistically compared by Tukey's test, where *p* values less than 0.001 were considered to be significantly different.

## Results

### Effects on plant growth and physiology

Water hyacinth growth was inhibited due to S-AgNP treatments in a concentration dependant manner, i.e. at 10 mg $L^{-1}$ concentration of AgNPs produced notable effects when compared to controls (Fig. 2a). In contrary, B-AgNPs treatment enhanced the growth of water hyacinth to their controls at 1 and 100 mg $L^{-1}$ concentrations. However, at 10 mg $L^{-1}$ dose a reduction in water hyacinth plant growth was noted (Fig. 2a). Change in plant height

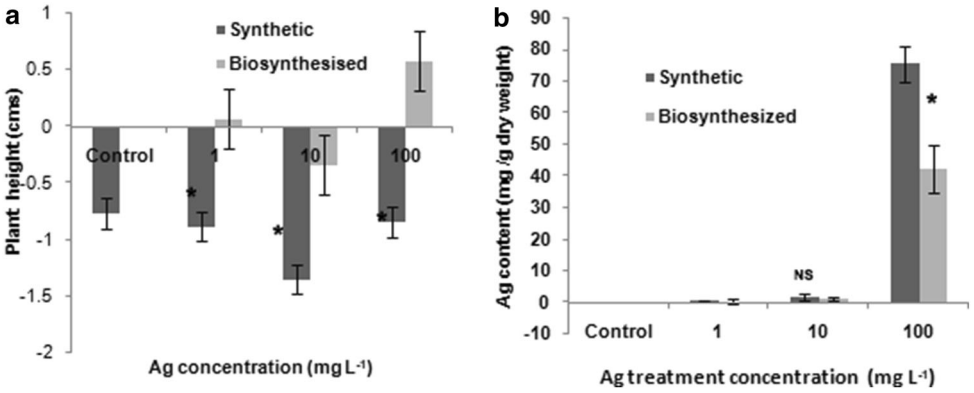


**Fig. 2** Effect of chemically synthesized (S-AgNPs) and biosynthesized silver nanoparticles (B-AgNPs) treatment on water hyacinth plants, **a** plant height and **b** silver content. *Bars* represents the mean ± SE. *Asterisks* indicate statistical significance at $p < 0.001$. *NS* not significant

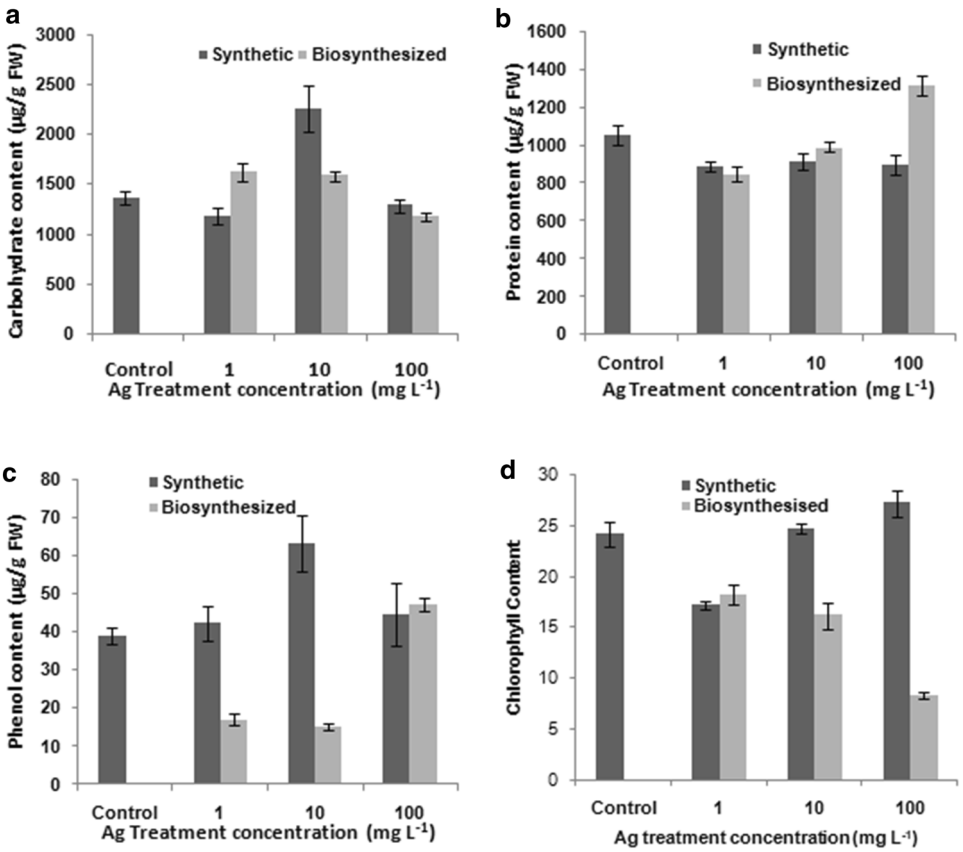


**Fig. 3** Effect of chemically synthesized and biosynthesized silver nanoparticles on the biochemical parameters of the water hyacinth plants **a** carbohydrate, **b** protein, **c** phenol, and **d** chlorophyll contents. *Bars* represents the mean ± SE. *Asterisks* indicate statistical significance at $p < 0.001$

directly denoted the plant growth and the negative values of plant height in Fig. 2a depict the reduced growth of water hyacinth plants due to nanoparticle treatment.

### Absorption and accumulation of silver nanoparticles

Absorption of AgNPs was evident by silver accumulation in water hyacinth plant tissues only at the fifth day of the nanoparticle exposure. Silver uptake by water hyacinth plants was found to be 0.9 and 25.048 mg $g^{-1}$ for S-AgNP treatments (Fig. 2a) and 0.35 and 13.9 mg $g^{-1}$ for B-AgNP treatments (Fig. 2b) 10 and 100 mg $L^{-1}$concentrations, respectively ($p < 0.001$). Surprisingly, B-AgNPs did not produce any phytotoxic effects in these plants, rather enhanced the plant growth. For 1 mg $L^{-1}$, there were no toxic effects observed due to minimal uptake of silver nanoparticles by water hyacinth plants.

### Effects on carbohydrate and protein contents

S-AgNP treatments increased the carbohydrate content at 10 mg $L^{-1}$ ($p < 0.001$, Fig. 2a), but did not show any effect on protein content, which was comparable to controls (Fig. 2b). On the other hand, B-AgNPs treatment increased the levels of carbohydrate content at 1 and 10 mg $L^{-1}$, respectively ($p < 0.001$, Fig. 2a). But, enhanced protein content was found only at concentration 100 mg $L^{-1}$ ($p < 0.001$, Fig. 2b).

### Effects on total phenol and chlorophyll content

S-AgNP treatments resulted in a quantitative increase in the amounts of total phenol contents at all the concentrations with a peak at 10 mg $L^{-1}$ ($p < 0.001$, Fig. 3c). Similarly, chlorophyll content was also increased in a concentration dependent manner (Fig. 3d). In contrary, treatment with B-AgNPs resulted in a decrease in phenol and chlorophyll content (Fig. 3c, d).

### Effect on antioxidative enzymes

Reactive oxygen species formation due to AgNP treatments was indicated by changes in antioxidative enzyme activities. S-AgNP treatments inhibited SOD activity at all the tested concentrations (Fig. 4a), albeit in a dose related manner ($p < 0.001$). Although, CAT activity was increased with all concentrations with their concurrent controls ($p < 0.001$, Fig. 4b), there were no dose related effects observed. The POD activity decreased with an increase in concentration in both the treatments when compared to

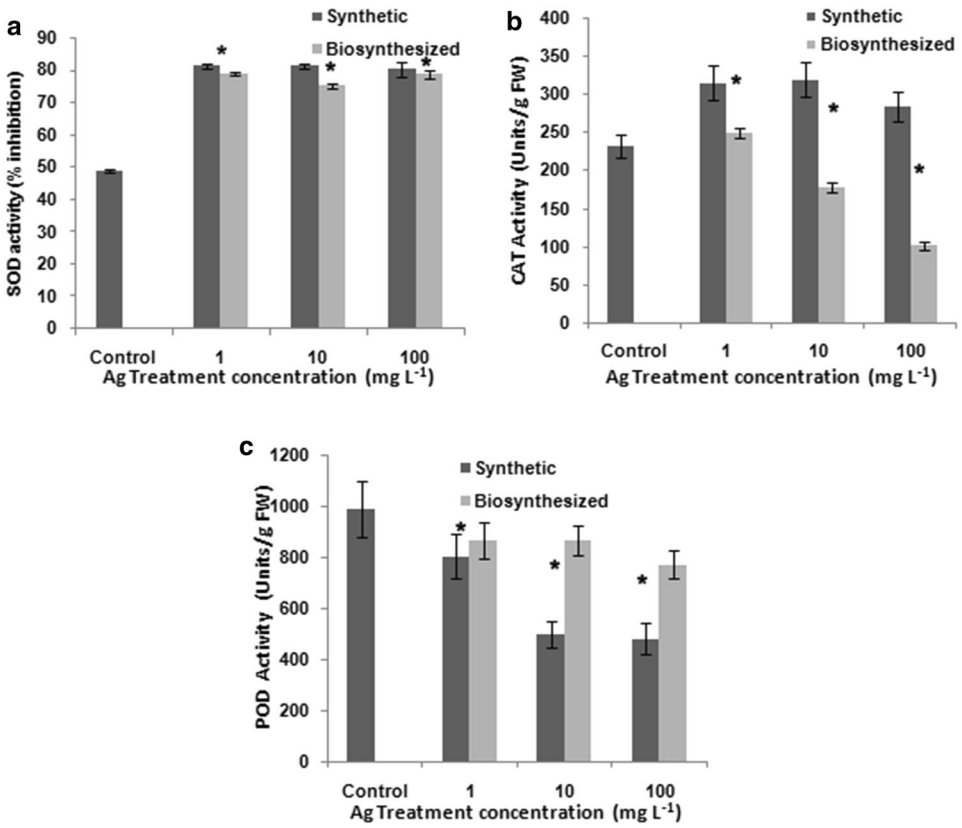


**Fig. 4** Changes in the antioxidative enzymes on treating *E. crassipes* plants with different concentrations of chemically synthesized and biosynthesized silver nanoparticles. **a** Superoxide dismutase, **b** catalase and **c** peroxidase activity. *Bars* represents the mean ± SE. *Asterisks* indicate statistical significance at $p < 0.001$. *NS* not significant

controls ($p < 0.001$, Fig. 4c). But the SOD activity was inhibited in all the concentrations of B-AgNPs (Fig. 4a). Surprisingly, CAT and POD activities were decreased with an increase in concentration when compared to control values indicating the lesser production of ROS in water hyacinth plants (Fig. 4b, c).

## Discussion

Effect of silver nanoparticles synthesised using two different methods was tested to study their impact on the growth and physiology of water hyacinth plants. Exposure of *E. crassipes* plants to the silver nanoparticles resulted in the uptake of the AgNPs. Thus the changes occurring in the *E. crassipes* plant metabolism may be attributed to the absorption of silver nanoparticles by the plants. This uptake of AgNPs by water hyacinth plants had affected the plant growth and produced physiological changes. Exposure of water hyacinth plants to chemically and biologically synthesized AgNPs revealed an increased plant height with B-AgNPs and decreased plant height in S-AgNPs treated plants. Reduction in growth of water hyacinth plants treated with S- AgNPs treatments may be due to the inhibition of metabolic reactions. There were only negligible effects found in water hyacinth plants when treated with biosynthesized AgNPs.

Toxic effects of AgNPs are mostly related to the surface coating, the aggregation state, and the release of dissolved silver. Kennedy et al. (2010) and Angel et al. (2013) reported that citrate-coated AgNPs were more toxic than PVP-coated AgNPs to fresh water organisms. This indicates that the type of coating affects the toxicity and dissolution. Zhao and Wang (2012) also reported different toxicities for *D. magna* exposure to lactate- and PVP-coated AgNPs due to the variations in the release of dissolved silver. According to manufacturer's instructions, S-AgNPs are stabilised with poly vinyl pyrrolidine (PVP), while the B-AgNPs are stabilised by the proteins and phenols present in the plant extract used for synthesis as reported earlier (Rajasekharreddy et al. 2010). In recent study, the Ag nanoparticles synthesised using different substrates also show varied response on plant growth (Unrine et al. 2012). The surface coatings of AgNPs could be one of the probable reasons for the changes that have occurred in the plant growth of water hyacinth plants. Also the increased growth of water hyacinth plants may be attributed to biomolecules (proteins and phenols) found around the NPs and also lesser dissolution due to surface coating.

Uptake and distribution limits of AgNPs in terrestrial plants such as *Brassica juncea* and *Medicago sativa* have been demonstrated earlier (Harris and Bali 2008). There was an increased AgNPs uptake in *M. sativa* than in *B. juncea*, along with increase of concentration and exposure time. Similar uptake was measured in the AgNPs treated water hyacinth plants using atomic absorption spectroscopy and was highest in the higher concentration tested. Growth of *Lemna* was reported to be completely inhibited at >100 mg $L^{-1}$ AgNPs and it is interesting that this result coincides with the observations made in the present work. This shows that aquatic plants may be more sensitive to nanosilver toxicity than the terrestrial plants studied previously (Kim et al. 2011). Though *E. crassipes* plants are known to be hyperaccumulators, there occurred a growth inhibition at the (1, 10 and 100 mg $L^{-1}$) tested concentrations of S-AgNPs. Application of metals on *E. crassipes* and *P. stratiotes* plants affected the root growth, development and relative growth rate (Odjegba and Fasidi 2007).

In earlier literature it is reported that the metal pollutants in water have deleterious effect on the biochemical aspects of plant life. Chlorophyll content, which acts as a biomarker of the photosynthetic activity of a plant, may be reduced when exposed to metals (Ouzounidou 1994). The chlorophyll contents in water hyacinth plants were enhanced with increase in S-AgNPs concentration and decreased when treated with B-AgNPs. These changes incurred can be related to the Ag uptake by *E. crassipes* plants. Similar observations were reported for chlorophyll content of *Chlamydomonas reinhardtii* exposed to AgNPs (Navarro et al. 2008). Decreased chlorophyll contents indicate disturbed chlorophyll synthesis which may have serious implications on the synthesis of organic food material. Also this might affect the carbohydrate synthesis which is clear from the altered carbohydrate and protein contents found in S-AgNPs treated *E. crassipes* plants. Reduction in carbohydrate content on treatment with B-AgNPs is perhaps the result of decreased photosynthesis. In contrast, a decrease in protein concentration can be attributed to both breakdown of existing proteins and reduced de novo synthesis. Such reductions in protein contents may act as bioindicator of metal stress in plants (Mane et al. 2011).

Phenols are secondary metabolites of plants whose levels are enhanced as a response to metal stress (Dudjak et al. 2004). Both the NP treatments had enhanced the total phenol contents in *E. crassipes* plants in this study. Phenols are also known to be involved in the antioxidant activity in plants growing under heavy metal stress. Phenols are oxidized by peroxidase and have a role in scavenging $H_2O_2$ molecules (Singh and Malik 2011). Induction of phenols was reported in buckwheat plants in response to nickel toxicity (Sytar et al. 2013). *Phaseolus vulgaris* plants treated with $Cd^{2+}$ had accumulated soluble and insoluble phenolics (Fuhrer 1982). Also in our previous studies with castor on S-AgNP treatments had enhanced the phenols and phenolic acid content (Jyothsna and Usha Rani 2013).

Previous studies have demonstrated that the absorption of metals by plants causes oxidative stress further leading to the formation of ROS in plant tissues (Singh et al. 2006). Formation of ROS leads to imbalance in scavenging mechanisms in plants further resulting in damage the cellular components of the organism (Matsumura et al. 2002; Mittler 2002; Nel et al. 2006). In *Lemna gibba* plants, the cytotoxic effects on plant growth and cellular viability were attributed to $Ag^{+}$ formed upon absorption of NPs by the plant cells. They suggest that these effects could lead to oxidative stress in plant cells resulting from the interaction of $Ag^{+}$ with proteins and/or enzymes (Oukarroum et al. 2013). It was previously illustrated that the production of ROS in *C. reinhardtii* cells resulted from exposure to Ag compounds (Navarro et al. 2008). Altered activities of the antioxidative enzymes indicate formation of ROS in *E. crassipes* plants which are scavenged by these enzymes.

Increased SOD activity was found in the plants treated with higher concentration of S-AgNPs. In water hyacinth plants treated with B-AgNPs, the activity increased initially and then decreased. It was also demonstrated in earlier reports, that in *E. crassipes* and *P. stratiotes* plants the application of heavy metals increased the activity of antioxidative enzymes in both species and their induction differs with metal treatment (Odjegba and Fasidi 2007). We presume that the increased SOD activity in the present work signifies the formation of ROS on AgNPs treatment.

An alternative approach of $H_2O_2$ destruction is through peroxidases as they have a higher affinity for $H_2O_2$ than CAT (Noctor and Foyer 1998). Peroxidase activity was decreased in both nano silver treatments in this study. The enhanced CAT activity in water hyacinth plant thus indicates an efficient detoxification of $H_2O_2$ than POD. Further, the regulation of CAT and POD suggest their predominant role as an antioxidative mechanism that enables water hyacinth plants to overcome stress caused by AgNPs. Earlier Krishnaraj et al. (2012) had reported that increase in CAT and POD activity found in *Bacopa monnieri* plants on B-AgNPs treatment implied less ROS formation resulting in low toxicity to plants. Also previous studies with AgNPs treatment on castor seedlings demonstrated altered activities of oxidative enzymes (Jyothsna and Usha Rani 2013).

In conclusion, the study of silver treatments on water hyacinth exposed to S-AgNPs showed reduced plant growth. The B-AgNPs had caused enhanced growth at the highest concentrations tested. The relationship between the induction of the antioxidative enzymes against ROS produced and its viability indicated the physiological alteration induced on water hyacinth plants are due to accumulation of Ag

nanoparticles. Our results clearly suggest that the silver ions from the S-AgNP suspensions could be a probable source of toxicity to plants when compared to B-AgNPs. Furthermore, it is apparent from the results that under these experimental conditions the production of ROS caused oxidative stress that was responsible for the decline in plant growth on silver nanoparticle exposure. Thus study on the aquatic plants gives clear picture of accumulation of the nanoparticles due to seepage of these particles into the aquatic ecosystems. Thus care has to be taken in disposing of the effluents of chemical nanoparticles and also this study supports the idea of replacing the chemically synthesized nanoparticles with biosynthetic nanoparticles which have potential applications but have less toxicity on the environment and non target organisms.

**Author contribution statement** PU designed research and finalised the manuscript. JY analysed the data prepared figures photos and manuscript carried out statistical analysis KSL and DD have conducted all the experiments.

**Acknowledgments** Authors are grateful to Dr. S. Chandrasekhar, Director, CSIR- Indian Institute of Chemical Technology, Hyderabad, India, for providing the facilities and Ministry of Environment and Forests, New Delhi for research grant to carry out the present research. One of the authors JY thanks CSIR, New Delhi, for Senior Research Fellowship.

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