Assessment of indole-3-acetic acid induced morpho-physiological responses and ROS scavenging in silver nanoparticles stressed *Oryza sativa* seedlings

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Abstract

Silver nanoparticles (AgNPs) are used in several commercial products, medical and diagnostic treatments, and as nano-pesticide and nano-herbicide. On release, it contaminates the environment and ultimately reaches the agriculture field, causes an alteration in the plant growth and development, whereas their negatives impacts could be restored by the exogenous application of indole-3-acetic acid (IAA). Therefore, the present study has been carried out to examine the potential role of IAA on the toxicity of AgNPs in Oryza sativa seedlings. AgNPs at both doses (200 and 500 µM) showed a remarkable decline in growth, photosynthetic pigments, and protein content. AgNPs also negatively affected the kinetics of photosystem II (PS II) photochemistry that leads to oxidative stress as confirmed by in- vitro and in-vivo visualization of superoxide radicle (SOR) and hydrogen peroxide (H2O2) that ultimately cause cell death. While the addition of IAA along with AgNPs mitigates the toxicity by increasing the photosynthetic pigment, protein content and it also repairs the kinetics of PSII photosystem and reduces the generation of reactive oxygen species. Therefore, this study concludes that IAA ameliorates the negative impacts caused by AgNPs and improves plant growth.

Keywords: Growth, photosynthetic pigment, protein, *in- vitro* and *in-vivo* ROS generation

1.0 Introduction

Rapid progression and advancement in the field of nanotechnology cause contamination risk in the environment. These nanoparticles are unique due to their small sizes (less than 100 nm), high reactivity and increased surface area. Therefore, use of nanoparticles in several commercial sectors is very common from the

last decades. Among many other metal nanoparticles, AgNPs are widely used nanoparticles due to its catalytic, superconducting, biocidal and antimicrobial properties (Ma et al., 2010; Stegemeier et al., 2015). Unconstrained use of AgNPs in industries and its direct release through water and waste channels, contaminate the agricultural land. Consequently, the plant grown in contaminated soils absorb AgNPs and excessively accumulate Ag ions which further leads to toxicity thereby decreased plant growth and productivity. AgNPs cause adverse impacts in plant growth by decreasing photosynthetic pigment kinetics of PS II photochemistry and enhancing the generation of reactive oxygen species that eventually increases level of oxidative stress (Dimkpa et al., 2013). Therefore, the mitigation approach is necessary to increase the plant growth, several phytohormones are used as an ameliorative agent, among which most important signal phytohormones is indole-3-acetic acid (IAA) an auxin contributor in root-shoot elongation (Ochoa et al., 2018). Several studies reported the role of IAA to increase the root growth, stem elongation tropism, adventitious root formation, and cell division under different stresses (Ochoa et al., 2017; He et al., 2018; Zhang et al., 2019; da Costa et al., 2018; Zhang et al., 2019). Therefore, the supplementation of IAA through different mechanism could mitigate the toxicity of AgNPs.

2.0 Materials and Methods

2.1. Plant and growth conditions

Seeds of *Oryza sativa* (Rice) were procured from a certified seed agency of Prayagraj, Uttar Pradesh. Seeds were surface sterilized through 2% sodium hypochlorite and soaked in DDW for 12 hr in dark. Afterwards, seeds were kept for germination. After 4-5 days, germinated seedlings were selected and grown in photosynthetically active radiation (PAR) of 300 µmol photons $m^{-2}s^{-1}$ and light-dark regime of 16:8 h with relative humidity of 60-70 % at 26 ±1 °C.

2.2. Treatment design and dose selection

Uniform size seedlings were selected and kept for acclimatization. After 7 days seedlings were treated with AgNPs and IAA alone and in combination. The experimental set-up were (i) Control (half strength Hoagland solution), (ii) 200 µM AgNPs, (iii) 500 µM AgNPs, (iv) IAA (5 µM), (v) 200 µM AgNPs + IAA, (vi) 500 µM

AgNPs + IAA. Treatments were changed following the three day interval and aerated regularly to avoid anoxia in root. All the parameters were examined after 7 days for growth of treated seedlings.

2.3. Growth behaviour and Protein content

The growth behaviour of treated and untreated seedlings were analysed in terms of fresh weight and protein by using the method of Lowry et al. (1951).

2.4. Total chlorophyll content

The Photosynthetic pigment i.e. Chlorophyll *a* and chlorophyll *b* **(**Chl *a* and Chl *b*) were assayed by using the method of Lichtenthaler (1987). Seedlings were homogenized in acetone and absorbance was taken at 663.2 and 646.5 nm respectively.

2.5. Activity of PS II

The activity of PS II photochemistry was examined in dark adopted leaves through FluorPen FP 100, Photon System Instruments, Czech Republic (Strasser et al., 2000)

2.6. *In-vitro* and *in-vivo* assessment of Oxidative stress

Reactive oxygen species (ROS), SOR ($O_2^{\bullet-}$) (Elstner and Heupel, 1976) and H_2O_2 (Velikova et al., 2000) accumulation was *in-vitro* assayed in leaf of treated and control seedlings. *In-vivo* ROS generation was also histochemically done by using the NBT and DAB stain, following the method of Frahry and Schopfer (2001) and Thordal-Christensen et al. (1997), further after staining and proper washing stained leaves were photographed with Nikon coolpixW150.

2.7. Statistical analysis

The data was statistically analyzed by the analysis of variance (ANOVA). Duncan multiple range test (DMRT) was performed for the significant differences among treatments at $P<0.05$ levels.

3.0 Results

3.1. Growth attributes and Protein content

The growth of treated and untreated seedlings was measured in terms of fresh weight (FW) (Fig. 1). The results showed that AgNPs at 200 and 500µM declined the plant FW by 24 and 40 % respectively as

compared to control. Whereas, addition of IAA in the medium increased plant FW by 19 %. While, supplementation of IAA along with AgNPs at both doses (200 and 500µM) reduce the loss and plant showed only 10 and 22% reduction in their fresh weight respectively in comparison to control.

The protein content of treated and untreated seedlings has been depicted in Fig 2. The results showed that AgNPs at both doses i.e. 200 and 500µM remarkably reduced the protein content by 29 and 44 % as compared to control seedlings. Whereas supplementation of IAA in AgNPs treated seedlings improved protein content by reducing the loss of damage and plant showed 11 and 25 % reduction at both doses.

3.3. Photosynthetic pigments

Photosynthetic pigments are necessary component for the Photosynthesis (Fig. 3). These pigments like chl *a*, significantly decreased under AgNPs treatment by 25 and 38 % and chl *b* by 21 and 31 % in dose dependent manner as compared to control seedlings. Whereas, addition of IAA alone in growth medium increased the pigment content chl *a* and chl *b* by 20 and 18 % respectively. However, addition of IAA along with AgNPs reduced the toxicity of AgNPs at both doses i.e. 200 and 500µM and seedlings showed only 8 and 12 % and 17 and 20 % reduction in chl *a* and chl *b* respectively.

3.4. PS II photochemistry

The efficiency of PSII photochemistry affected under AgNPs treatment in concentration dependent manner (Fig. 4). Parameters related to chlorophyll *a* fluorescence, like quantum yield of primary photochemistry (Phi_P_0), yield of electron transport per trapped excitation (Psi_$_0$), quantum yield of electron transport (Phi_E_0), and performance index of PS II (PI_{ABS}) significantly declined. While differing to these kinetics parameters the energy flux parameters like ABS/RC, TR_0/RC, DI_0/RC and ET_0/RC showed noticeable enhancement in comparison to control seedlings.

3.5. *In-vitro* and *in-vivo* assessment of oxidative stress biomarkers (SOR and H_2O_2) content

Biochemical analysis of SOR and H_2O_2 content has been depicted in Fig 5. The graph clearly exposed that under both the tested dose of AgNPs, SOR and H_2O_2 content enhanced by 32 and 60 % and 36 and 70% respectively as compared to control treated seedlings. Whereas, addition of IAA in the growth medium

reduced the ROS generation and showed reduction by 12 and 20 % in SOR and H_2O_2 content respectively, while along with AgNPs, IAA reduced the generation of oxidative stress markers as it showed 10 and 8% SOR and H_2O_2 generation with lower dose of AgNPs. Similar trend has been observed with higher dose of AgNPs.

To verify the biochemical analysis of oxidative stress markers, *in-vivo* histochemical analysis was also performed. The SOR and H_2O_2 content in leaves reacted with NBT and DAB and gave blue and brown coloration respectively. The intensity of these coloration was much intensified in AgNPs treated seedlings in dose dependent manner. Whereas supplementation of IAA with AgNPs reduced the intensity which showed less production of SOR and H_2O_2 content.

4.0. Discussion

The current study shows the mitigating behaviour of IAA against AgNPs toxicity in crop plant *Oryza sativa*. The growth of plants was significantly affected under the toxicity of AgNPs toxicity in concentration dependent manner. The exposure of AgNPs on plants increased the accumulation of Ag ion that caused prominent reduction in photosynthetic pigment as well as affected the kinetics of PSII photochemistry. Altered PSII affects the photosynthetic process and causes electron leakage, and therefore increase the oxidative stress, which eventually reduce plant growth. Similar to our study, previous studies also corroborates that AgNPs caused adverse impacts on aquatic plant *Lemna gibba*, seagrass (*Halophila stipulacea*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) plant (Oukarroum et al., 2013; Mylona et al., 2020; Fayez et al., 2017; Dimkpa et al., 2013). The photosynthetic pigments are essential component of photosynthetic mechanism which also found reduced in *O. sativa* seedlings under AgNPs toxicity. The reduction in the photosynthetic pigment might be due to replacement of co-factors responsible for the biosynthesis of chlorophyll molecule (Mishra et al., 2016), liking to this decline with the altered kinetics of photochemistry of PSII (Stoeva et al., 2005). Similar to our results Wang et al. (2016) also reported the altered PSII kinetics under stress condition. Furthermore, alteration in the kinetics of PS II also cause decline in the efficiency of Phi_E_o and Psi_$_o$, while enhanced flux ratios might be due to condensed number and size of active reaction centre (Singh et al. 2019). The altered pigment content and kinetics of PS II cause electron

leakage leads to oxidative stress. Increased production of ROS induces cell death (Baxter et al., 2014). Generation of ROS is also significantly proved by the *in-vivo* analysis. Similar result was also reported by Mylona et al. (2020) related to the damages caused because of increased oxidative stress indicated by increased level of H_2O_2 and SOR under AgNPs stress in *Halophila stipulacea. Corroborate to our* result with previous studies on soybean and rice plant stressed under AgNPs resulted into a decreased biomass and elevated level of malondialdehyde and H_2O_2 contents (Li et al., 2017).

Addition of IAA against AgNPs treatments improves the plant growth, protein content and decline the ROS generation i.e. $O_2^{\bullet-}$ and H_2O_2, as shown in *in-vitro* and *in-vivo* results. Addition of IAA balances Ag content in plants which might be a reason for increased pigment content and improved PSII efficiency of *O. sativa* seedlings. Similar to our study Gangwar and Singh (2011), Bashri and Prasad (2016) suggested the mitigating behaviour of IAA against heavy metal stress. Exogenously applied IAA reduces the ROS production by activating antioxidant defense system, which balance the redox status of cell and improves the plant growth (Gangwar and Singh, 2011; Bashri and Prasad, 2016)

Conclusion

The present study concludes that AgNPs significantly declined the growth of *O. sativa* due to Ag accumulation as well as decrease pigment content and enhanced oxidative stress markers i.e. SOR and H_2O_2. Whereas addition of IAA mitigates the toxic impacts and shielded the plant from the AgNPs toxicity. IAA increases the protein content, photosynthetic pigment content as well as reduce the oxidative stress. Therefore, this study suggests the potential role of IAA against the toxicity caused by AgNPs in *Oryza sativa* seedlings.

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Conflicts of interest

Authors declare no conflict of interest

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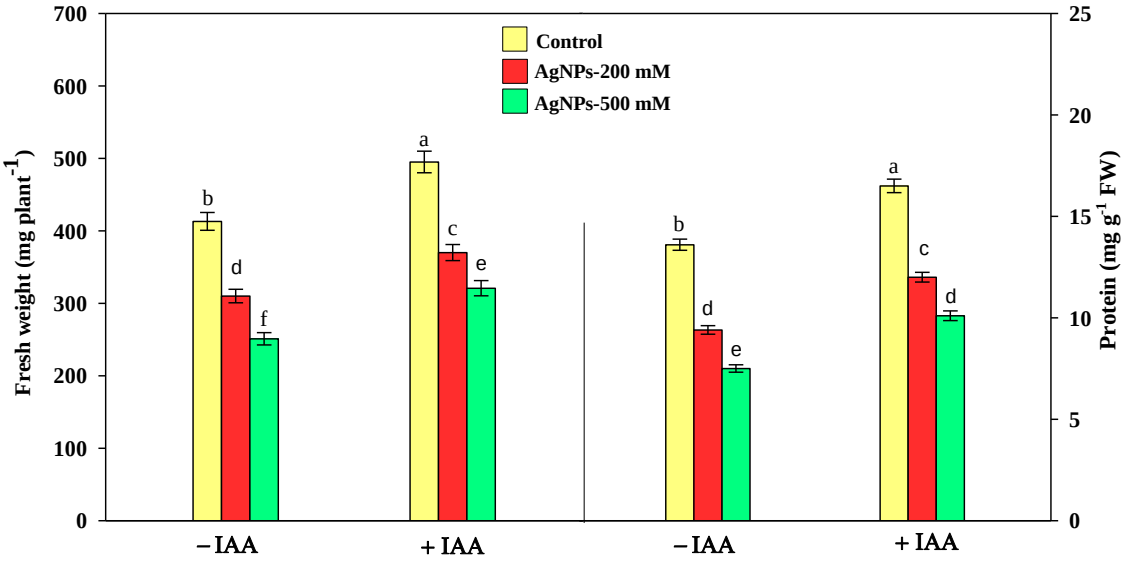

Fig 1: Growth parameters (Fresh weight and Protein) of *O. sativa* seedlings grown in the presence of AgNPs alone or in combination of IAA. Data are means ± standard error of three replicates. Bars with different letters show significant differences at $p<0.05$ according to the Duncan's multiple range test.

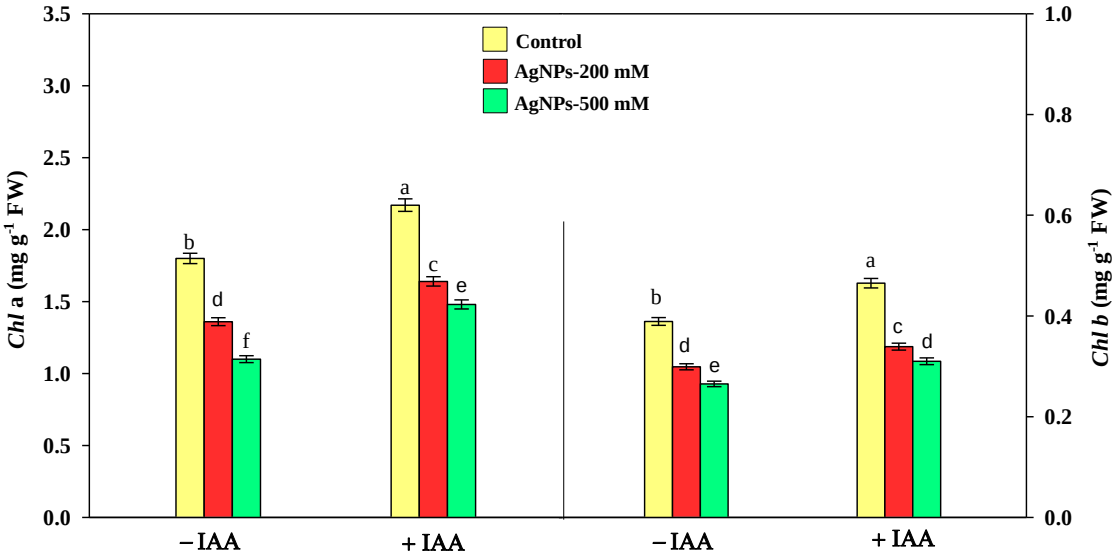

Fig 2: Photosynthetic pigment of *O. sativa* seedlings grown in the presence of AgNPs alone or in combination of IAA. Data are means ± standard error of three replicates. Bars with different letters show significant differences at $p<0.05$ according to the Duncan's multiple range test.

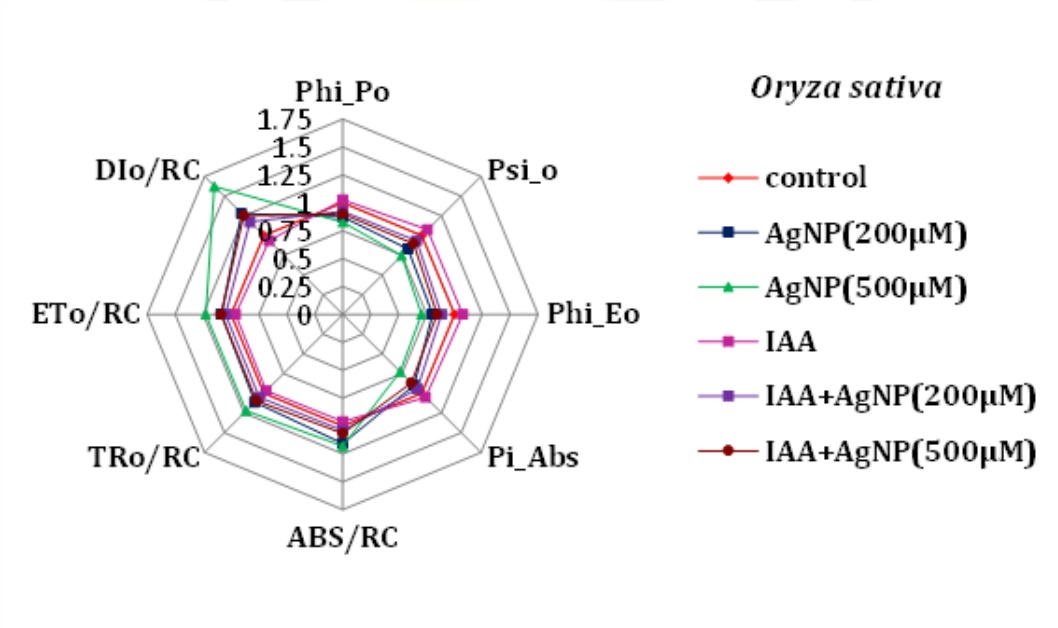

Fig 3: Radar graph showing the altered kinetics of PS II in *O. sativa* seedlings in the presence of AgNPs alone or in combination with IAA.

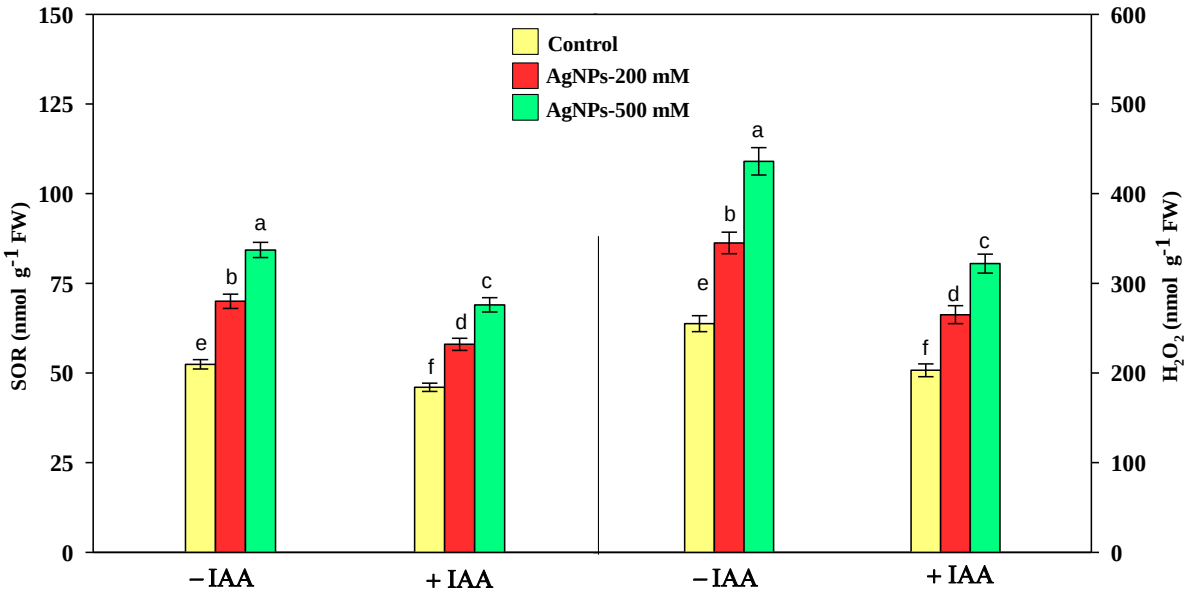

Fig 4: (A) SOR and **(B)** H_2O_2 accumulation of *O. sativa* seedlings grown in the presence of AgNPs alone or in combination of IAA. Data are means ± standard error of three replicates. Bars with different letters show significant differences at $p<0.05$ according to the Duncan's multiple range test.

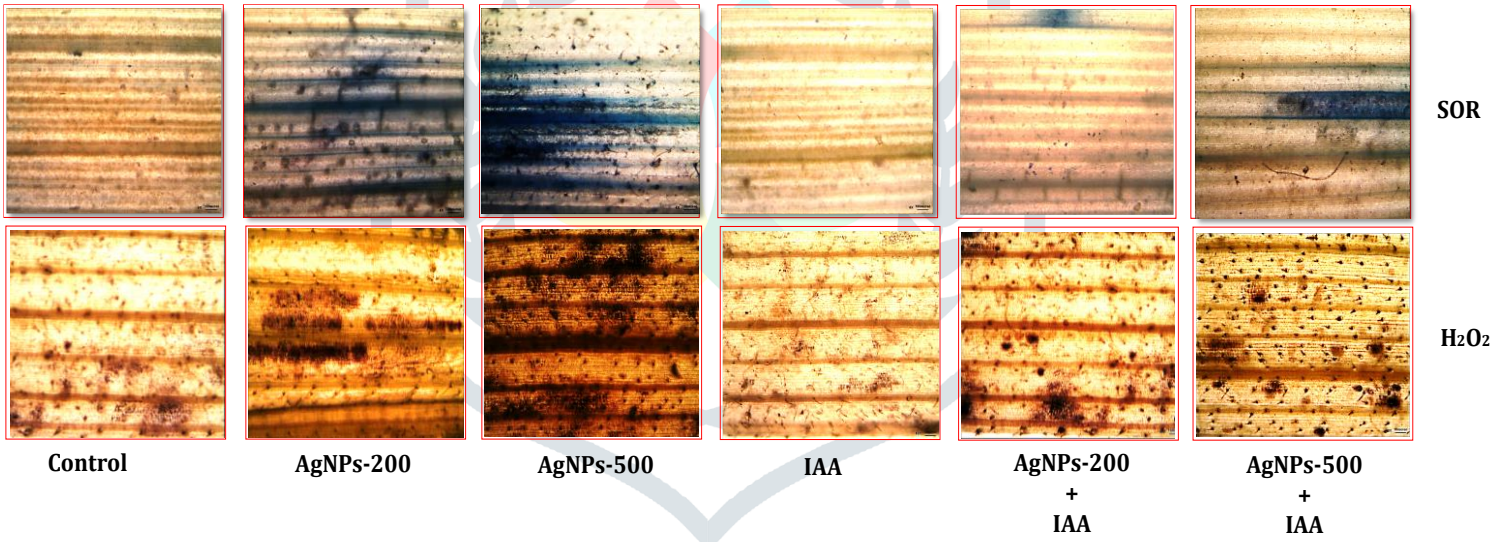

Fig 5: *In-vivo* production of SOR and H_2O_2 of *O. sativa* seedlings grown in the presence of AgNPs alone or in combination of IAA.