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Research Article

Pretreatment with salicylic acid and nitric oxide alleviates silver nanoparticles toxicity in medicinal *Hypericum perforatum* plants

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Abstract

Since the use of seed priming agents to reduce the potential toxicity of AgNPs has not been explored, we evaluated the combined effects of 0.1 mM salicylic acid (SA) and 0.1 mM nitric oxide (NO) on responses of *Hypericum perforatum* plants to Ag-induced stress. The results of growth parameters together with measurement of malondialdehyde (MDA) revealed that exposure to AgNPs or $AgNO_3$ caused more toxicity, which was closely associated with the over–accumulation of ROS. However, SNP (NO) and SA addition successfully mitigated adverse impact of AgNPs on *H. perforatum* seedlings, which was associated with the higher synthesis of chlorophyll *b* pigments and proline. In addition, a further increase in antioxidants (phenolic compounds) was observed in NO-primed plants under AgNPs-induced stress, which was concomitant with the high level of SOD activity. We concluded that NO minimized the inhibitory effects of AgNPs stress by increasing the phenolic compounds as well as by regulating accumulation of Ag, ROS and antioxidants. To sum up, exogenous NO plays a key role in enhancing its resistance to Ag stress in physiological base, thereby improving the growth and phenolic compounds production (chlorogenic acid, quercetin and rutin) of *H. perforatum* plant.

Keywords: Chlorogenic acid, Exogenous nitric oxide, *Hypericum perforatum*, Salicylic acid, Silver nanoparticle, Toxicity

Introduction

While the Food and Agriculture Organization and the World Bank are also promoting nanoparticles use in agriculture (Mahmood *et al*., 2021), their load in agricultural soils is expected to rise. Increasing use of NPs in agricultural inputs like fertilizers and pesticides causes the input of NPs in agricultural soils which can affect soil fauna and flora and thereby the associated agroecosystem services (Seleiman *et al*., 2020). To sum up, nanotechnology and nanoparticles (NPs) have positive and negative aspects and environmental challenges as well (Zhang *et al*., 2018; Szollosi *et al*., 2021). Since the use of NPs leads to the alterations in growth, biological function, gene expression, and development of plants (Szollosi *et al*., 2021), specific research on NPs toxicity in plants is urgently needed to use nanotechnology safely (Zulfiqar *et al*., 2019).

While silver nanoparticles (AgNPs) have been used as a novel nanopesticide and nanofungicides (Zhang *et al*., 2018), the toxicity of AgNPs to various species has been shown in a number of studies (Lee *et al*., 2012; Wang *et al*., 2013; Noori *et al*., 2020). AgNPs at toxicity levels caused adverse effects on gene expression, growth and development (Wang *et al*., 2020; Rizwan *et al*., 2021) via increasing in ROS formation and lipid peroxidation (Li *et al*., 2017; Zhang *et al*., 2018).

In this regard, plant priming by chemical compounds has been proposed an effective method in enhancing plant tolerance to AgNPs stress by regulating accumulation of Ag and ROS, and antioxidants (Tripathi *et al*., 2017). NO (SNP) protects pea seedlings against nano silver toxicity via enhancing the activities of antioxidant enzymes and proline accumulation, in parallel to increasing NO release levels in the roots (Amooaghaie *et al*., 2018). Additionally, exogenous application of SA increased heavy metal stress tolerance by improving antioxidative defense system, osmolyte accumulation and ionic homeostasis (Sharma *et al*., 2020).

To better understand the effects of AgNPs, we investigated plant physiological responses upon exposure to AgNPs in comparison to the silver nitrate ($AgNO_3$). Although the potential toxicity of AgNPs has been studied to some extent recently, such kind of studies is rare in soils. Moreover, the use of SA and NO to diminish the potential toxicity of AgNPs has not been explored. We hypothesized that these two molecules would reduce the negative impact of AgNPs and $AgNO_3$ on *Hypericum perforatum* plants, as well as improve the removal efficiency of these toxicants from soils. The present findings provide important knowledge to design strategies that minimize the negative impact of AgNPs and $AgNO_3$ on crops.

Materials and methods

Plant material and treatments: Seeds of *Hypericum perforatum* were sown in top of the cylindrical plastic pots filled with perlite and then watered with 500 ml of half-strength Hoagland solution containing $NH_4H_2PO_4$

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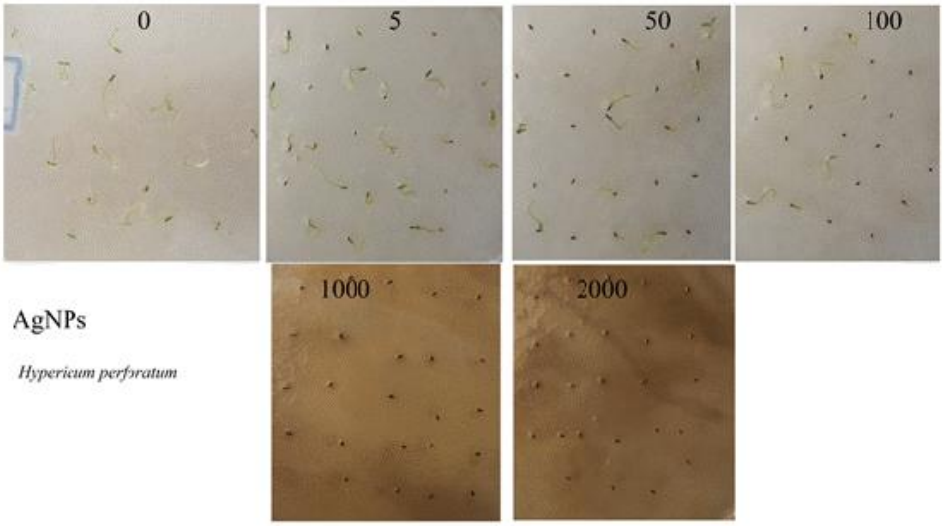

Fig. 1. Effect of NO and SA on the germination of *Hypericum perforatum* seeds. Seeds were exposed to 0, 0.05, 0.1, 0.5, 1, 5 and 10 mM SNP or SA for 12 h.

(115.03 g/l), KNO_3 (101.10 g/l), $Ca(NO_3)_2.4H_2O$ (236.15 g/l), $MgSO_4.7H_2O$ (246.47 g/l), KCl (3.728 g/l), H_3BO_3 (1.546 g/l), $MnSO_4.H_2O$ (0.845 g/l), $ZnSO_4.7H_2O$ (0.575 g/l), $CuSO_4.5H_2O$ (0.125 g/l), $H_3MoO_4.H_2O$ (0.09 g/l), FeEDTA (9.31 g/l). Pots were 14 cm in diameter and 45 cm in depth. For priming treatments, *Hypericum perforatum* seeds were soaked in aerated solutions of sodium nitroprusside (SNP, as a NO donor) and salicylic acid (SA) for 12 h. The concentrations of NO and SA were chosen according to the effect of different NO (0.05, 0.1, 0.5, 1, 5 and 10 mM SNP) and SA (0.05, 0.1, 0.5, 1, 5 and 10 mM SA) concentrations on *Hypericum perforatum* seed germination as well as on seedling growth in a preliminary study, which showed that 0.1 mM NO and SA priming significantly promoted seed germination and seedling growth (Fig. 1). Sixteen weeks after sowing, when the plants were about 10 cm tall, the pots were irrigated with 1000 ppm AgNPs or $AgNO_3$ solution dissolved in tap water for 21 days (Ag treatment). The Ag nanoparticle product (AgNPs) was procured from the reliable company entitled "Iranian Nanomaterials Pioneers, Mashhad, Iran". The physicochemical traits of this nano-product are as follows: Purity: 99.99 %; APS: 5-8 nm; SSA: 25-42 m^2/g; Color: black; Morphology: spherical; True Density: 10.9 g/cm^3. Equivalent silver salt ($AgNO_3$) was purchased from Sigma−Aldrich and utilized as a bulk control. The concentration of silver nanoparticle was chosen according to the effect of different AgNPs (5, 50, 100, 1000 and 2000 ppm) concentrations on seed germination as well as on seedling growth in a preliminary study (Fig. 2). At 100 ppm AgNPs, the germination percentage halved as compared with that of the control and it was selected as a semi-lethal (LC50) concentration (Zhang *et al.*, 2008) of AgNPs for further experiments (data no showed). The control plants were irrigated with the nutrient solution without Ag, NO and SA. Plants were maintained in a greenhouse under 16/8 h day/night cycle, day/night temperature of 25-30/19-21 ºC, relative humidity of 60-65 % and daily photon flux density of about 350-400 µmol m^{-2} s^{-1} throughout the experimental period.

After 21 days of Ag treatment, plants were harvested for morphological and physiological analysis. After determination of fresh weight (FW), leaves were dried out for 48 h at 70 °C for determination of dry weight (DW). For the latter physiological analysis, samples were stored immediately in liquid N_2 until assay.

Assay of chlorophylls and carotenoids: For determination of leaf concentration of chlorophyll and carotenoids, samples were homogenized in the methanol according to Lichtenthaler and Wellburn (1983). The homogenate was filtered, and after centrifugation at 1000 rpm for one minute, supernatants were used for determination of pigments. Photosynthetic pigments were measured spectrophotometrically at 400–700 nm.

Determination of total protein, soluble sugars and proline: The extraction and quantification of total soluble proteins were carried out by the method of Bradford (1976) using a commercial reagent (Sigma) and BSA (Merck) as standard. Proline was calculated as described by Bates *et al*. (1973). Leaf samples from each group were homogenized in 3% (w/v) sulphosalycylic acid at 4 °C and the homogenate was centrifuged at 3,000g for 20 min. Mixture was boiled for 1 h in water bath after addition of acid ninhydrin and glacial acetic acid. The tube was cooled over crushed ice, and then absorbance at 520 nm was determined. Standard curve was created using proline (Sigma). Soluble sugars concentrations were determined according to the method of Quentin *et al.* (2015). Leaf tissues were homogenized with 2.5 mL 80% ethanol in a water bath for 2 h at 30 ºC. After centrifugation at 3,000 g for 10 min, the supernatants were subjected to soluble sugars analysis by anthrone-sulfuric reagent at 630 nm.

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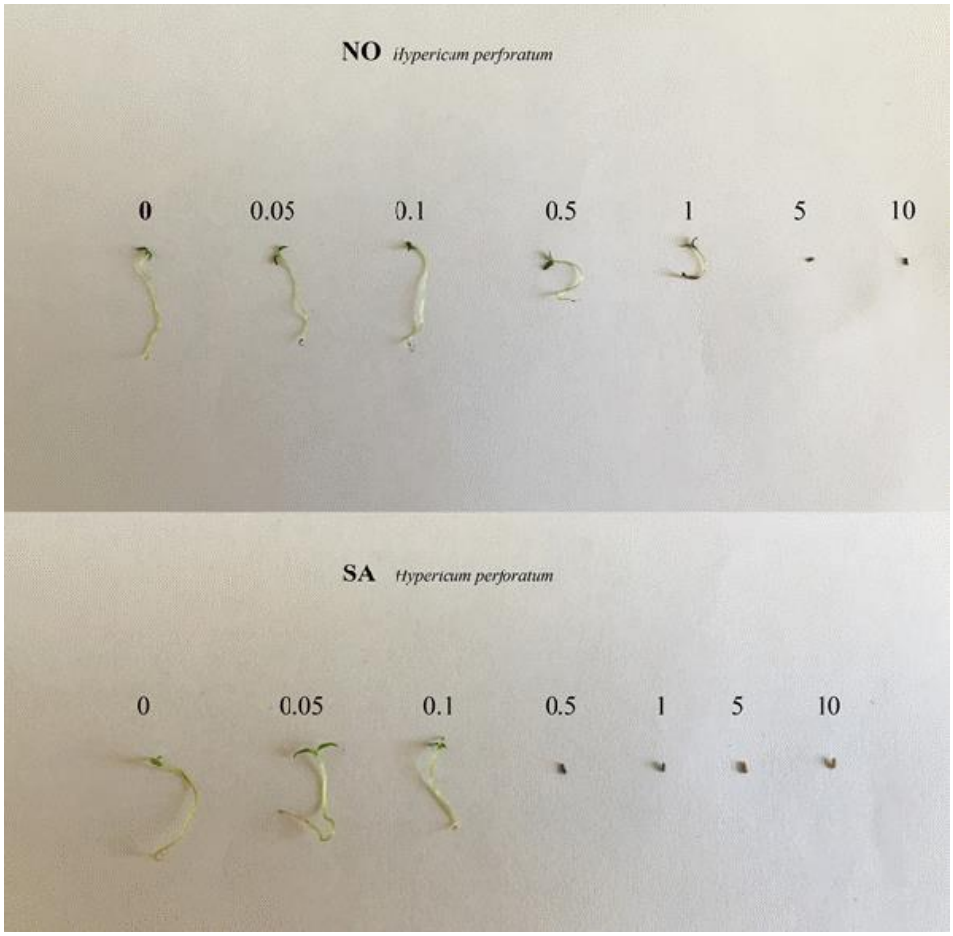

Fig. 2. Effect of AgNPs on the germination of *Hypericum perforatum* seeds. Seeds were exposed to 0, 5, 50, 100, 1000 and 2000 ppm AgNPs for 48 h.

Glucose (Sigma) was used for production of a standard curve.

Assay of phenylalanine ammonia-lyase (PAL) activity and related metabolites: To estimate PAL activity, leaf sample was ground in 50 mM sodium phosphate buffer (pH 7.0) containing 2 % (w/v) polyvinylpolypyrrolidon (PVPP), 2 mM EDTA, 18 mM β-mercaptoetanol and 0.1 % (v/v) Triton X-100. Formation of cinnamic acid was determined by spectrophotometry at 290 nm according to modified method of Zucker (1965). One unit (U) of PAL activity was expressed as the amount of the enzyme that produced 1 nmol cinnamic acid per h. Total phenolic content was evaluated by the method of Velioglu *et al.* (1998). Gallic acid was used for constructing the standard curve. Results were defined as mg gallic acid (GA) per gram of the fresh weight. The total flavonoid content was measured using a standard curve of quercetin and expressed as mg quercetin equivalent (QE)/100 g extract. For the determination of anthocyanin content leaves were homogenized in ice bath with 3 ml HCl-methanol solvent (1: 99, v: v) and then, the supernatant was filtered after allowing the samples to stand in darkness at 5°C for 24 h. The amount of anthocyanin was calculated from the absorbance at 550 nm.

Assay of antioxidant enzymes activities and related metabolites: Determination of the activity of antioxidant enzymes and concentration of related metabolites were done according to the methods described by Habibi and Hajiboland (2012). Fresh samples were ground in the presence of liquid nitrogen and measurements were undertaken using spectrophotometer. Superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated according to the method of Giannopolitis and Ries (1977). Enzyme was extracted in 25 mM HEPES (pH 7.8) with 0.1 mM EDTA and the supernatant was added to the reaction mixture containing 0.1 mM EDTA, 50 mM Na_2CO_3 pH 10.2, 13 mM methionine, 63 µM nitroblue tetrazolium chloride (NBT), 13 µM riboflavin. One unit of SOD was defined as the amount of enzyme which produced a 50% inhibition of NBT reduction under assay conditions. For the determination of catalase (CAT, EC 1.11.1.6) activity, samples were homogenized with 50 mM phosphate buffer (pH 7.0) and assayed spectrophotometrically by following the degradation of H_2O_2 at 240 nm according to the method of Simon *et al.* (1974). Reaction medium contained 50 mM phosphate buffer, 10 mM H_2O_2 and one unit represented 1 µmol

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H_2O_2 decomposed min^{-1}. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following the decrease in absorbance at 290 nm according to the method of Boominathan and Doran (2002). The reaction mixture contained 50 mM phosphate buffer (pH 7), 0.2 mM EDTA, 0.5 mM ascorbic acid and 50 µg BSA. One unit represented 1 µmol ascorbate oxidized min^{-1}. Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid according to methods described elsewhere (Habibi and Hajiboland, 2012). **Quantification of NO concentration:** Nitric oxide concentration was measured using the modified method described by Wu *et al*. (2016). Leaves were homogenized in 50 mM cool acetic acid buffer (pH 3.6, containing 4% zinc diacetate). This extract was centrifuged at 10,000 g for 15 min at 4 °C, and the supernatant was collected. The pellet obtained by centrifugation was resuspended in cool acetic acid buffer, and then centrifuged. The two supernatants were combined and 0.1 g of charcoal was added. After filtration, the filtrate was added to the reaction mixture consisting of Greiss reagent, and incubated at room temperature for 30 min. Absorbance was monitored at 540 nm. The NO concentration was calculated using a standard curve plotted with known concentrations of $NaNO_2$.

Determination of Ag content: The sample was digested with 5 ml of a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) (v/v, 4:1) at 130 ºC for 1 hr. After cooling, 5 ml of concentrated hydrochloric acid (HCl) was added and incubated at 115 ºC for 20 min. The resulting solution was diluted in distilled water, and the Ag content was estimated with an Inductively-Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, INTEGRA XL2, GBC; Australia).

HPLC analysis: The leaf extract (0.5 g) was prepared using methanol (5 ml) as a solvent and filtered prior to HPLC analysis. The supernatant was centrifuged at 3000g for 3 minutes, and then filtered prior to HPLC analysis. For the calibration curve, the stock solutions of the identified phenolic compounds were prepared with methanol to obtain a 1 mg/ml concentration, and the calibration curves for standard samples were constructed by plotting the peak area of the identified phenolic compounds against their concentrations through dilution of each stock solution in methanol to six concentrations (0.78 ppm, 1.58 ppm, 3.12 ppm, 6.25 ppm and 25 ppm). The HPLC analysis was carried out using a Agilent 1290 high-performance-liquid-chromatography (HPLC) system (Santa Clara, CA, USA) with an Agilent 1290 diode-array detector (DAD) according to Sinrod *et al*. (2019). Separation was achieved on a 25 cm×4.6 mm Eurospher 100-5 C18 analytical column with pre-column provided by Knauer (Berlin, Germany). Data acquisition and integration were performed with EZchrom Elite software. A 20 µl sample of the methanol extract in leaves was injected into an HPLC column through a 3900 Smartline Autosampler injector equipped with a 100 µL loop. Separation was carried out using 0.02% trifluoroacetic acid in water (elution A) and methanol (elution D). The total running time was 55 minutes at a flow rate of 0.5 ml/min, and the oven temperature was at 20 ºC. The quantification of each phenolic acids peak was done using the calibration curves in the range of 0.78-25 ppm, and their equations were measured.

Experiments were done in complete randomized block design with 4 replications (pot), 2 plants per pot. Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test ($P<0.05$).

Results and discussion

Exogenous NO pretreatment improved growth of *H. perforatum* under Ag stress: In the present study, the root dry weight was not significantly affected by single SA and NO or by combination of SA+NO under both $AgNO_3$ and AgNPs treatments. However, the results showed that both $AgNO_3$ and AgNPs significantly declined the shoot dry of *Hypericum perforatum* (Fig. 3), similar to previous observations, which confirmed that AgNPs decreased the growth of many plant species (Wang *et al*., 2020). In many plant species, AgNPs caused adverse effects on cell division and/or cell elongation and growth and development (Zulfiqar *et al*., 2019; Wang *et al*., 2020). However, the growth inhibition under AgNPs was alleviated by NO priming. Our results are consistent with the findings of Amooaghaie *et al*. (2018) for *Brassica nigra*, who reported that the NO improved plant growth under AgNPs stress.

Both shoot and root Ag contents were significantly enhanced by both $AgNO_3$ and AgNPs treatment, and increased further by $AgNO_3$ treatment (Table 1). The highest accumulation of Ag was obtained in SA+NO pretreated leaves under $AgNO_3$ stress. In fact, pretreatment with NO tended to increase Ag translocation from the roots into photosynthetic organs under AgNPs stress while saving proper growth.

Exogenous SA and NO pretreatment affected Chl*b*/Chl*a* and compatible solute content under silver ions and silver nanoparticles stress: Under AgNPs treatment, SA+NO application caused an increase in Chl*b*/Chl*a* levels (Fig. 4). In fact, the highest Chl*b* and carotenoids contents have a role in the light harvesting and photoprotection processes via xanthophyll cycle (Aragon–Gastelum et al., 2020; Habibi, 2020; Habibi, 2021). Thus, our results revealed that *H. perforatum* plants primed with SA+NO exhibited higher synthesis of chlorophyll *b* pigments to overcome the impaired electron transfer imposed by AgNPs. In one of these studies, Alamri *et al*. (2018) demonstrated that exogenous salicylic acid can suppress chlorophyll degradation in wheat plants under heavy metal stress. Moreover, treatments with NO also resulted in the up-regulation of chlorophyll contents in *Pisum sativum* seedlings under silver nanoparticles

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Table 1. Effect of SA and NO addition on the Ag concentration (µg g^{-1}) within *Hypericum perforatum* plant under $AgNO_3$ or AgNPs-stressed conditions. Data of each row within each parameter indicated by the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

Treatment	Shoot Ag^+ concentration	Root Ag^+ concentration
Control	0.011±0.004 c	0.07±0.01 c
$AgNO_3$	2.14±0.17 b	2.93±0.17 a
$AgNO_3$+SA+NO	5.47±0.29 a	3.65±0.47 a
AgNPs	1.61±0.10 b	1.96±0.21 b
AgNPs+SA+NO	1.53±0.10 b	2.08±0.23 b

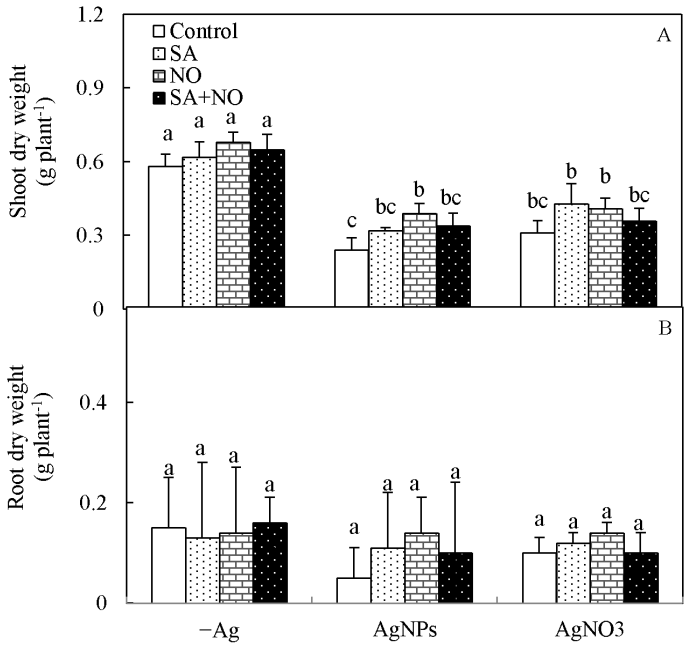

Fig. 3. Effect of SA and NO addition on the shoot and root dry weight of *Hypericum perforatum* under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

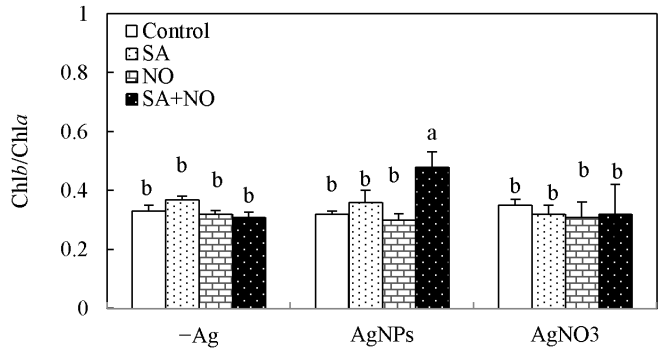

Fig. 4. Effect of SA and NO addition on the chlorophyll and carotenoids content in *Hypericum perforatum* leaves under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

(AgNps)-induced phytotoxicity (Tripathi *et al*., 2017).

The results revealed that total proline contents were significantly improved by AgNPs and $AgNO_3$ treatments (Fig. 5). While total soluble sugars

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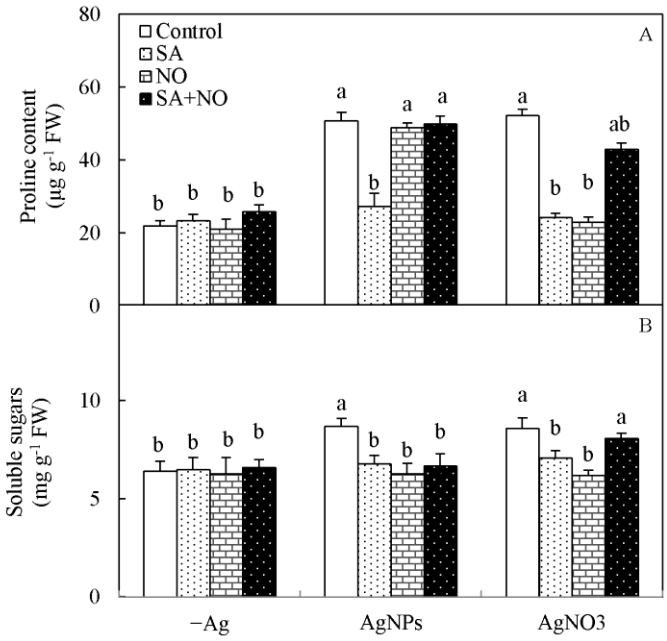

Fig. 5. Effect of SA and NO addition on the soluble sugars (mg g^{-1} FW) and proline (µg g^{-1} DW) content in *Hypericum perforatum* leaves under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

concentration was enhanced by AgNPs treatment, it was not influenced by exposure to SA or NO priming, compared with control. Under $AgNO_3$ treatment, while soluble sugars concentration was not affected by exposure to SA or NO alone, it was significantly increased by the combination of SA and NO. It has been reported that a severe increase in sugar contents as well as in proline contents was found in rice (*Oryza sativa* L.) seedlings treated with AgNPs (Nair and Chung, 2014). Sugars, as important signaling molecules, are known to accumulate during stress in leaves (Wingler *et al*., 2000; Zhang *et al*., 2018). In this study, a significant increase in the accumulation of proline and soluble sugars was recorded upon exposure to $AgNO_3$ and AgNPs, which possibly participated in the stress tolerance of *H. perforatum* plants.

Priming enhanced phenolic accumulations under Ag stress: Under non-stress conditions, phenolic and flavonoids contents were not affected by exposure to SA and NO priming (Fig. 6). Phenolic and flavonoids contents were significantly enhanced by AgNPs application, which was associated with a marked increase in PAL activity. Compared with the control, the activity of PAL was not significantly affected by single SA and NO or by combination of SA+NO under both $AgNO_3$ and AgNPs treatments.

Since phenolic compounds are important in the inhibition of lipid peroxidation and radical scavenging (Oh *et al*., 2009) as well as in the plant resistance to environmental stresses (Blasco *et al*., 2013; Su *et al*., 2018), in the present study, we showed an increase in phenol and flavonoids content as well as in PAL activity in AgNPs-exposed plants. We assessed the main phenolic acids in the methanol extract of leaves using HPLC analysis (Table 2). With exogenous application of SA, chlorogenic acid content was significantly increased as compared with the control (Table 2). Addition of SNP further increased the level of quercetin and rutin compounds under AgNPs-induced stress. Chlorogenic acid (CGA) is a type of polyphenol and one kind of secondary metabolites, which has anti-inflammatory, anti-oxidative and anticancer properties (Gengmao *et al*., 2015). With exogenous application of NO alone under AgNPs-induced stress, increases in the accumulation of quercetin and rutin compounds may be another active protective mechanism employed by *H. perforatum* upon AgNPs stress (Zhang *et al*., 2018; Noori *et al*., 2020).

Priming stimulated the antioxidant defense systems and NO levels during Ag stress: The superoxide dismutase (SOD) activity in the AgNPs-stressed plants remained unchanged, but an increase was observed under combined AgNPs and priming by SA treatment alone (Fig. 7). Also, the CAT activity in the primed AgNPs-stressed plants remained unchanged. A similar observation was recorded for ascorbate peroxidase (APX) activity pattern in plants exposed to AgNPs and combined priming. Similar to Laxa *et al*. (2019) and Hasanuzzaman *et al*. (2020), in the present study, the accumulation of phenolic was attendant with the high level of SOD activity in primed plants under AgNPs stress, compared with the control (the control

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Table 2. Effect of SA and NO addition on the content of phenolics (mg ml^{-1}) in leaves of *Hypericum perforatum* under AgNPs-stressed conditions. Data of each row within each parameter indicated by the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

Treatment	Chlorogenic acid	Quercetin	Rutin
Control	0.55±0.01 ^{e}	3.00±0.41 ^{d}	16.5±0.96 ^{e}
SA	2.72±0.12 ^{a}	4.64±0.49 ^{ab}	13.3±0.19 ^{e}
NO	1.46±0.22 ^{d}	3.36±0.32 ^{cd}	27.1±1.79 ^{c}
SA+NO	1.57±0.07 ^{d}	3.72±0.13 ^{c}	23.2±1.24 ^{d}
AgNPs	0.68±0.03 ^{e}	4.29±0.40 ^{ab}	36.1±1.07 ^{b}
AgNPs+SA	2.32±0.16 ^{b}	3.94±0.07 ^{bc}	37.1±1.76 ^{b}
AgNPs+NO	1.88±0.19 ^{c}	4.80±0.11 ^{a}	47.8±2.09 ^{a}
AgNPs+SA+NO	1.99±0.14 ^{c}	3.51±0.13 ^{cd}	27.3±0.94 ^{c}

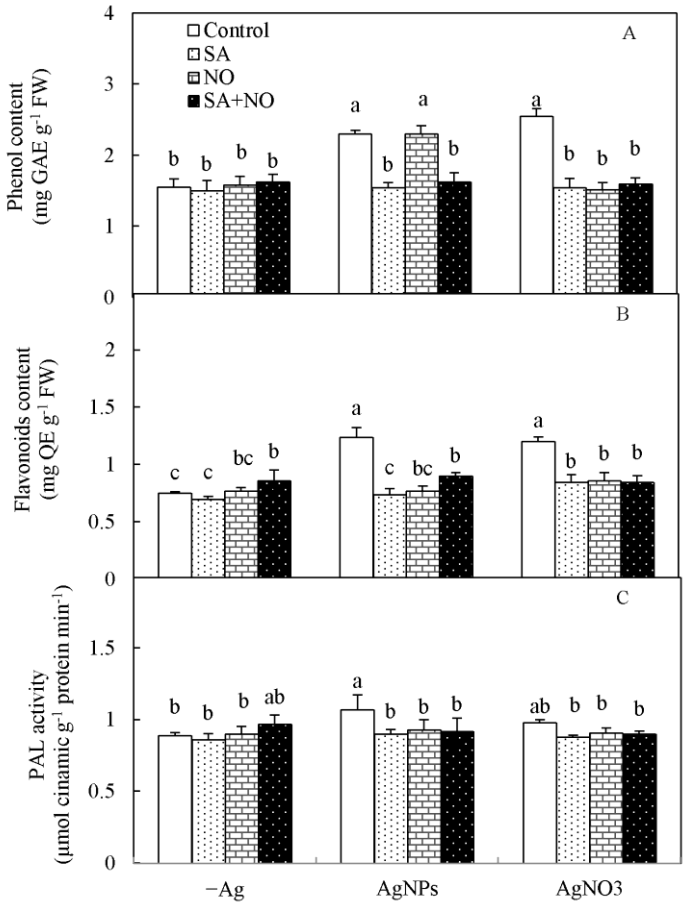

Fig. 6. Effect of SA and NO addition on the total phenol and flavonoids content, and the activity of phenylalanine ammonia-lyase (PAL) in *Hypericum perforatum* leaves under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

plants were irrigated with the nutrient solution without Ag, NO and SA). In fact, we suppose that SA and NO alleviated the inhibitory effects of AgNPs stress on the structure of the photosynthetic apparatus by increasing the total phenolic and flavonoids contents as well as by regulating accumulation of ROS and via enhancement in the activities of CAT and SOD enzymes (Kotapati et al., 2017).

Furthermore, both $AgNO_3$ and AgNPs considerably increased the H_2O_2 and MDA levels, as an indicative of lipid peroxidation, and severely damaged membrane structures (Fig. 8). Here, the higher MDA levels showed potentially significant membrane damage as a function of Ag exposure. Similar results were obtained by Mo *et al*. (2021), who reported that Ag at high concentration (100 mg/L) exhibited obvious inhibition on cell viability, which was due possibly to the induction of ROS accumulation. However, priming with SA and NO greatly reduced MDA content and repaired membrane structures, contributing to the better induction of

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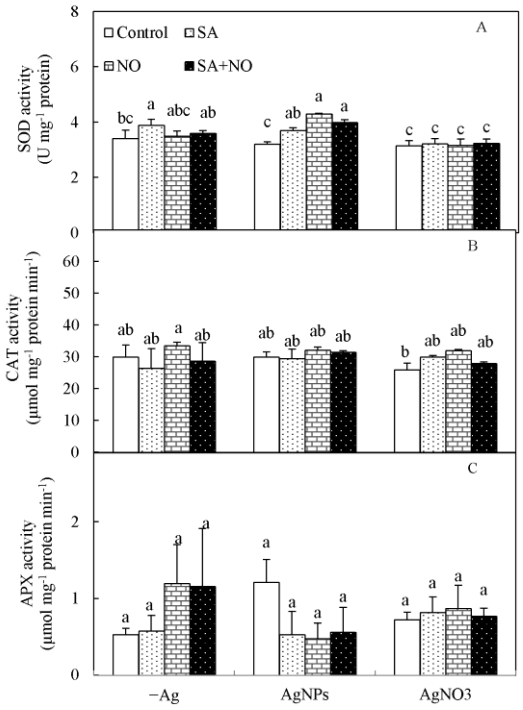

Fig. 7. Effect of SA and NO addition on the activity of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in *Hypericum perforatum* leaves under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

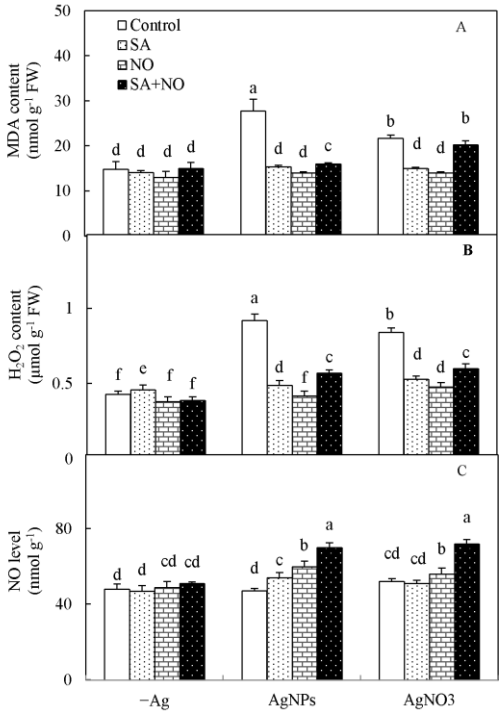

Fig. 8. Effect of SA and NO addition on the concentration of nitric oxide (NO), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) in *Hypericum perforatum* leaves under $AgNO_3$ or AgNPs-stressed conditions. Bars indicated with the same letter are not significantly different (P<0.05, Tukey test). Values are the mean ± SD (n=4).

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antioxidative enzymes activities in response to AgNPs stress. The present results were in agreement with previous, which revealed that pretreatment with NO alleviated salt-induced inhibitory effects in *Pisum sativum* seedlings (Tripathi *et al*., 2017) and *Brassica nigra* (Amooaghaie *et al*., 2018) via modulation of the antioxidative mechanism involved in removing ROS.

Interestingly, in this study, NO levels were enhanced to a higher extent in response to combined priming (SA+NO) than other treatments, indicating that SA probably influenced NO accumulation. However, future work will need to to be done with this respect. Increase in endogenous NO content following exogenous NO treatment was consistent with the findings of Balotf *et al*. (2018), which reported that exogenous NO caused an increase in leaf NO content in *Triticum aestivum* plants.

Conclusion

We showed that both $AgNO_3$ and AgNPs adversely decreased growth, due to enhanced level of Ag and significant lipid peroxidation. However, SA and SNP addition successfully decreased the levels of MDA and consequently mitigated adverse impact of Ag on *H. perforatum* seedlings. Strong amelioration of Ag-induced stress was achieved under NO priming. These findings provide valuable information for development of sustainable strategies in order to reduce the negative impacts of AgNPs and $AgNO_3$ on crops.

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