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Research article

Salicylic acid and jasmonic acid restrains nickel toxicity by ameliorating antioxidant defense system in shoots of metallicolous and non-metallicolous *Alyssum inflatum* Náyr. Populations

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ABSTRACT

The presence of heavy metals in the soils is undoubtedly one of the prime abiotic stresses in the world. There are a considerable amount of plant yield losses because of heavy metal stress. The goal of this study was to assess the morphological, physiological and biochemical changes in *Alyssum inflatum* Nyár. populations upon exposure to different levels of nickel (Ni) (0, 100, 200, 400) µM, salicylic acid (SA) (0, 50, 200) µM and jasmonic acid (JA) (0, 5, 10) µM. Results showed that there were no considerable interpopulation differences, including the shoot Ni concentrations. Reversing the effects of Ni, SA and JA decreased due to Ni accumulation in both populations. By increasing the levels of Ni stress, the fresh weight (FW) of shoot decreased, whereas the application of SA + JA elevated the FW of the shoot in NM plants. Also, SA + JA mitigated Ni oxidative effects by reducing H_2O_2 concentration in both populations. The results revealed that the exposure of both M and NM plants to high Ni concentration increased superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) activities compared to control in both populations. Conversely, APX activity was inhibited in NM plants. Furthermore, SA and JA treatments reversed the detrimental effects of Ni on carotenoid content and reduced the content of proline in plants exposed to Ni stress. All the above suggests that SA and JA confer tolerance to Ni stress in two population of *A. inflatum* via several mechanisms.

1. Introduction

In recent years, rapid industrialization has a drastic contribution to environmental pollution by releasing remarkable quantities of toxic metals, involving Nickel (Ni) to the environment (Rizwan et al., 2018). However, Ni is just one of the many ubiquitous trace metals that enter into the environment from both natural and anthropogenic sources (Mir et al., 2018). Ni is essential as a trace element in the majority of plant species when present in lower concentrations, e.g. 0.05–10 mg kg^{-1} dry weight (Chen et al., 2009). Also, it is naturally present as a significant structural constituent of some of the metalloenzymes as ureases, glyoxalases (family I), peptide deformylases, methyl Co-M reductases, hydrogenases and a few superoxide dismutases (SOD) (Sirhindi et al., 2015). Furthermore, the excess of Ni accumulation in plant tissues causes toxic effects by the formation of reactive oxygen species (ROS) or deficiency of other essential elements as a result of their similar chemical properties (Vatansever et al., 2017; Yamaguchi et al., 2017). However, since Ni is a non-redox-active metal, indirectly bringing about the formation of ROS. So, it enhances the amount of ROS leading to oxidative stress. Actually, to avoid oxidative stress, plants have antioxidative defense mechanisms, including a number of antioxidant enzymes like; SOD, catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and non-enzymatic anti-oxidant compounds such as proline and carotenoids which act as free radical scavengers (Chen et al., 2009; Yadav et al., 2016).

Salicylic acid (SA) is an endogenous plant hormone with phenolic nature (Hayat et al., 2010), that plays important role as a signaling molecule in physiological processes such as photosynthesis, in increasing antioxidative enzymes system, proline metabolism as well as increasing plant tolerance to biotic and abiotic stresses (Horváth et al., 2007; Kotapati et al., 2017). Several reports indicate that SA can improve the deleterious effect of heavy metals on subjected plants (Zhou et al., 2009; Ahmad et al., 2011; Gu et al., 2018).

Jasmonic acid (JA), its methyl ester, and Methyl jasmonate (MeJA)

Abbreviations: APX, Ascorbate peroxidase; CAT, Catalase; FW, Fresh weight; JA, Jasmonic acid; M, Metallicolous; Ni, Nickel; NM, Non-metallicolous; POD, Peroxidase; SA, Salicylic acid; SOD, Superoxide dismutase

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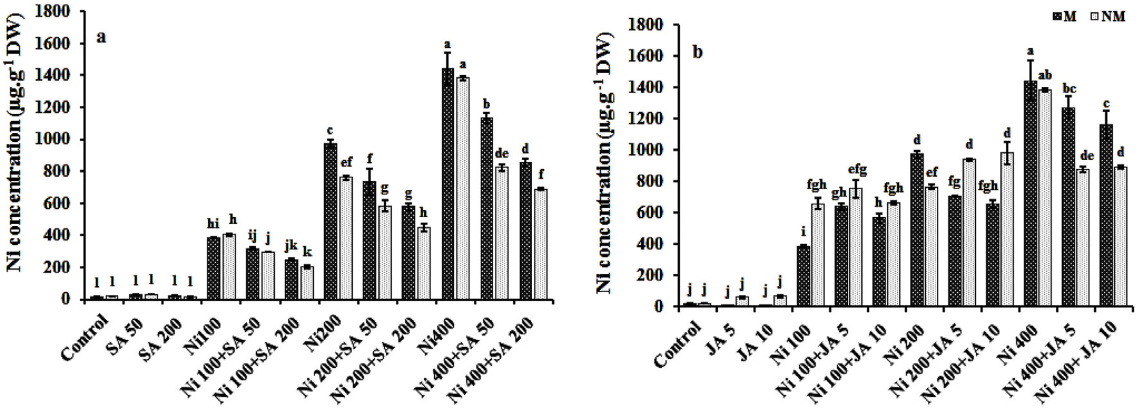

Fig. 1. The effect of SA (a) and JA (b) on uptake and accumulation of Ni in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (μM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

are a class of plant hormones adjusting many processes of the plant growth, and development in plants (Mahmood et al., 2012; Qiu et al., 2014). Furthermore, it has been shown that endogenous JA level increases in plants under various stress conditions (Bankaji et al., 2014; Kaya and Doganlar, 2016). The ameliorative effects of JA on the toxic effects of heavy metals was also reported through the increased accumulation of osmolytes, carotenoids and increased antioxidative defense (Dar et al., 2015). Furthermore, some studies have provided evidence for the synergistic effects of JA with other plant hormones for the regulation of plants growth and development (Dar et al., 2015; Avalbaev et al., 2016). JA-SA cross-talk is the most crucial cross-talk for increasing plant immunity against biotic stress (pathogens and insects). According to the best of our knowledge, no reports have examined the interactive effect of JA and SA in plants under heavy metal stress, although there are limited reports of other abiotic stresses such as salinity (Faghih et al., 2017; Farhangi-Abriz and Ghassemi-Golezani, 2018).

Alyssum inflatum Nyár. is an endemic plant of both serpentine and non-serpentine soils, which mostly populated on serpentine soils and is considered metallophyte (Ghasemi and Ghaderian, 2009; Ghasemi et al., 2014). As already pointed out, *A. inflatum* is a Ni hyperaccumulator plant (accumulates > 1000 µg g^{-1} Ni in leaves) which belongs to Brassicaceae family growing mainly in the western provinces (Kurdistan and Kermanshah) of Iran (Ghaderian et al., 2007; Van der Ent et al., 2013; Ghasemi et al., 2014).

This study was carried out to analyze, for the first time, the mechanism of beneficial effect of different concentrations of SA and JA on two geographically close populations of *A. inflatum* [metallicolous (M) and non-metallicolous (NM)] growing on two different substrates and serpentine and non-serpentine soils, and exposed to different Ni concentrations.

2. Materials and methods

2.1. Plant growth and treatment

The seeds of *A. inflatum* (M) were collected from serpentine soils at Marivan, the region in western Iran (35∘13.625′N; 46∘27.184′E) (Ghasemi et al., 2009). Also, *A. inflatum* (NM) were collected from non-serpentine soils of Shahu district (34°56′47″N; 46°27′41″E) between Ravansar and Paveh road, Kermanshah, Iran. Experiments were conducted by using a solution culture method on an inert substrate of perlite and sands (2:1) in a 450 ml pot. Eight seeds were sown in each pot with 3 replicates for each treatment (collectively in 216 pots and each pot emerged in a designed plastic bucket) and, routinely buckets were filled with 300 ml tap water. After 15 days, the water was replaced with modified 1/10-strength Hoagland's nutrient solution (pH 5.8) containing 1 mM Ca $(NO_3)_2$, 0.1 mM KH_2PO_4, 0.5 mM $MgSO_4$, 0.5 mM KNO_3, 0.2 µM $CuSO_4$, 0.2 µM $ZnSO_4$, 0.2 µM $MnSO_4$, 10 µM H_3BO_3, 0.1 µM Na_2MoO_4, 2 µM $MnCl_2$ and 0.01 mM FeEDDHA (ferric ethylenediamine-di-2-hydroxyphenylacetate). Subsequently, the pots were transferred to an environmentally controlled CG 72 Conviron Chamber (25 °C/20 °C, 16 h/8 h light/dark, ~140 µmol m^{-2} s^{-1} photosynthetic photon flux) and nutrient solution of all the pots was simultaneously changed every 5 days. Then after 45 days, pots were separately and interactively treated with different concentrations of Ni (0, 100, 200, 400) µM, SA (0, 50, 200) µM and JA (0, 5, 10) µM (treated with 36 different treatments). In every 7 days of treatment, Ni and SA were mixed in Hoagland's nutrient solution, and JA was sprayed on the leaves until drips formation. Three weeks after exposure, the plants were harvested and cut to shoots and roots and the fresh weight (FW) of shoots was measured. Then, the shoots were stored at −70 °C for further analysis.

2.2. Nickel concentration measurement

Ni concentration was determined according to the following procedure. Dried shoot samples were ground, homogenized and acid digested with 2 ml of 65% nitric acid overnight at room temperature, followed by heating at 90 °C for 4–5 h. After cooling, 1 ml H_2O_2 was added to the solution and heated again at 90 °C for 20 min or until clear. The final volume was adjusted by using distilled water. Finally, the concentrations of Ni were measured by atomic absorption spectrophotometry (AAS, Shimadzu model 6200).

2.3. Determination of hydrogen peroxide concentration

H_2O_2 levels were measured according to Sergiev et al. (2000) method. 0.5 g of tissue samples were extracted with 5 ml of 0.1% (w/v) cooled trichloroacetic acid (TCA) and centrifuged at 12000 × *g* for 15 min at 4 °C. The aliquot (0.5 ml) of supernatant was added to 0.5 ml potassium phosphate buffer (200 mM, pH 7.0) and 1 ml potassium iodide (KI) (1 M). The H_2O_2 content was analyzed based on the absorbance of the supernatant at 390 nm by using a standard curve.

2.4. Protein and enzyme extraction

Freshly frozen leaf tissues (0.1 g) were homogenized in 1 ml of cold 50 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP), and vortexing for 30 s. Then, the homogenates were centrifuged for 15 min at 13,000 × *g* at 4 °C, and the supernatant was used for the enzymes activity assays. The protein contents in the supernatants were determined as described by

Table 1

Effect of exogenous application of SA + JA on the FW, Ni concentration, proline and carotenoid content criteria in two populations of *A. inflatum* treated by different Ni levels.

Carotenoid (mg. g^{-1} FW)		Proline (μg. g^{-1} DW)		Ni concentration (μg. g^{-1} DW)		FW ($g.plant^{-1}$)		Treatments
NM	M	NM	M	NM	M	NM	M	
6.31 ± 0.06^{f-i}	5.07 ± 0.13^{mno}	12.2 ± 0.8^{j}	7.91 ± 0.74^{o}	19.3 ± 1.40^{q}	15.7 ± 1.60^{q}	1.56 ± 0.02^{j}	2.27 ± 0.05^{e}	Control
7.00 ± 0.13^{b-e}	4.42 ± 0.12^{pq}	11.6 ± 0.38^{kl}	5.36 ± 0.73^{tu}	56.2 ± 0.80^{pq}	18.0 ± 2.50^{q}	1.14 ± 0.003^{l-o}	2.11 ± 0.009^{fg}	(SA 50 + JA 5) μM
6.57 ± 0.11^{ef}	4.45 ± 0.14^{pq}	12.5 ± 0.38^{ij}	5.66 ± 0.64^{stu}	74.2 ± 12.2^{opq}	4.90 ± 3.10^{q}	1.08 ± 0.01^{op}	1.71 ± 0.01^{i}	(SA 50 + JA 10) μM
7.24 ± 0.06^{b}	3.85 ± 0.10^{r}	13.1 ± 0.43^{h}	4.40 ± 0.62^{v}	267 ± 22.0^{jkl}	148 ± 4.10^{no}	1.30 ± 0.004^{k}	2.44 ± 0.02^{cd}	(Ni 100 + SA 50 + JA 5) μM
6.64 ± 0.11^{def}	4.11 ± 0.18^{qr}	12.5 ± 0.33^{ij}	6.03 ± 0.97^{qrs}	265 ± 13.3^{kl}	328 ± 5.20^{h-k}	0.92 ± 0.005^{rs}	2.28 ± 0.04^{e}	(Ni 100 + SA 50 + JA 10) μM
6.37 ± 0.09^{fgh}	4.34 ± 0.01^{pqr}	12.7 ± 0.33^{h}	5.19 ± 0.75^{u}	333 ± 17.2^{h-k}	288 ± 6.10^{i-l}	1.03 ± 0.006^{op}	2.07 ± 0.02^{fg}	(Ni 200 + SA 50 + JA 5) μM
6.70 ± 0.28^{c-f}	4.04 ± 0.03^{qr}	14.1 ± 0.25^{g}	6.91 ± 0.54^{p}	719 ± 7.60^{gh}	392 ± 7.20^{gh}	1.20 ± 0.008^{l}	2.68 ± 0.01^{b}	(Ni 200 + SA 50 + JA 10) μM
6.68 ± 0.09^{def}	4.62 ± 0.13^{op}	14.8 ± 0.41^{f}	7.51 ± 0.91^{o}	982 ± 2.30^{a}	600 ± 8.90^{e}	1.15 ± 0.02^{lmn}	2.43 ± 0.02^{d}	(Ni 400- SA 50 + JA 5) μM
5.85 ± 0.2^{ijk}	4.99 ± 0.22^{no}	16.0 ± 0.43^{e}	7.92 ± 0.81^{o}	806 ± 63.8^{b}	429 ± 9.90^{bc}	1.09 ± 0.005^{nop}	2.87 ± 0.01^{a}	(Ni 400 + SA 50 + JA 10) μM
6.50 ± 0.09^{efg}	5.79 ± 0.05^{jkl}	12.1 ± 0.42^{jk}	5.64 ± 0.85^{stu}	70.3 ± 11.0^{opq}	64.2 ± 10.2^{pq}	0.87 ± 0.03^{s}	1.92 ± 0.01^{h}	(SA 200 + JA 5) μM
7.18 ± 0.49^{bc}	5.32 ± 0.25^{lmn}	11.4 ± 0.25^{l}	6.22 ± 0.67^{qr}	19.1 ± 1.20^{q}	68.2 ± 11.9^{pq}	0.95 ± 0.001^{qr}	2.29 ± 0.008^{e}	(SA 200 + JA 10) μM
7.34 ± 0.43^{b}	5.72 ± 0.14^{jkl}	16.6 ± 0.41^{d}	5.67 ± 0.46^{stu}	164 ± 8.10^{mn}	243 ± 12.5^{l}	1.12 ± 0.002^{mno}	2.05 ± 0.03^{g}	(Ni 100 + SA 200 + JA 5) μM
6.91 ± 0.14^{b-e}	6.02 ± 0.21^{g-j}	16.0 ± 0.43^{e}	6.50 ± 0.8^{pq}	105 ± 7.20^{nop}	380 ± 13.4^{gh}	1.19 ± 0.03^{lm}	2.34 ± 0.09^{e}	(Ni 100 + SA 200 + JA 10) μM
7.23 ± 0.18^{b}	5.94 ± 0.08^{h-k}	19.2 ± 0.46^{c}	5.91 ± 0.64^{rs}	105 ± 7.20^{nop}	356 ± 14.9^{ghi}	1.20 ± 0.03^{l}	1.90 ± 0.02^{h}	(Ni 200 + SA 200 + JA 5) μM
6.94 ± 0.11^{b-e}	5.67 ± 0.05^{jkl}	20.5 ± 0.48^{b}	5.78 ± 0.5^{rst}	499 ± 47.2^{f}	487 ± 15.2^{f}	1.02 ± 0.004^{pq}	1.90 ± 0.01^{h}	(Ni 200 + SA 200 + JA 10) μM
8.02 ± 0.22^{a}	5.92 ± 0.006^{h-k}	20.1 ± 0.44^{b}	9.15 ± 0.83^{n}	421 ± 4.20^{fg}	669 ± 16.5^{de}	1.07 ± 0.01^{k-o}	2.13 ± 0.02^{f}	(Ni 400 + SA 200 + JA 5) μM
7.07 ± 0.04^{bcd}	5.50 ± 0.04^{klm}	21.9 ± 0.46^{a}	10.6 ± 0.68^{m}	345 ± 25.8^{g-j}	822 ± 17.3^{b}	0.95 ± 0.009^{qr}	2.51 ± 0.01^{c}	(Ni 400 + SA 200 + JA 10) μM

The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

Bradford (1976). The bovine serum albumin (BSA) was used as the standard protein.

2.5. Measurement of antioxidant enzymes activity

SOD activity was measured according to Giannopolitis and Ries (1977). The reaction solution (3 ml) contained 50 μl protein extract, 0.2 mM riboflavin, 1 mM NBT, 13 mM methionine, 10 mM EDTA and 50 mM phosphate buffer (pH 7.8). One unit of SOD activity was defined as the amount of enzyme that inhibits the NBT photo-reduction by 50%.

CAT activity was assayed by the decomposition of H_2O_2 according to Aebi (1983). The reaction mixture (1 ml) consisted of 50 mM potassium phosphate buffer (pH 7) and, 30 mM H_2O_2. The reaction was started by adding 100 ml protein extract to 900 ml reaction solution. After enzyme addition, the reaction was monitored by following the decomposition of H_2O_2 at 240 nm ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$). Finally, CAT activity was expressed as U. mg^{-1} protein.

POD activity was measured according to the method of Chance and Maehly (1955). The assay mixture contained 200 mM phosphate buffer (pH 7), 1% guaiacol, 1 mM H_2O_2, and 100 μl enzyme extract. The increase in absorbance owing to the oxidation of guaiacol to tetraguaiacol ($\varepsilon = 26.6\ mM^{-1}cm^{-1}$) was monitored at 470 nm. POD activity was expressed as U. mg^{-1} protein.

By using Nakano and Asada (1981) method, the procedure of APX activity was measured. The reaction mixture (3 ml), contained 50 μl enzyme extract, 50 mM potassium phosphate buffer (pH 7), 1 mM EDTA, 5 mM ascorbic acid and 1 mM H_2O_2. APX activity was assayed as a decrease in absorbance at 290 nm of ascorbate and enzyme activity was calculated by using an absorbance coefficient for ascorbic acid of ($\varepsilon = 2.8\ mM^{-1}cm^{-1}$). APX activity was expressed as U. mg^{-1} protein.

2.6. Proline concentration

Proline concentrations in the shoots were determined spectrophotometrically as described by Bates et al. (1973). Dry shoot samples (0.1 g) were homogenized in 10 ml 3% (m/v) aqueous sulfosalicylic acid and, the homogenates were centrifuged at 1700 × *g* for 10 min. Then, 0.5 ml of the supernatant was transferred to a test tube, 0.5 ml of acetic acid and 1 ml acid ninhydrin was added and incubated for 1 h at 100 °C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 1 ml toluene, while mixed thoroughly by vortex. The absorbance of the upper phase was determined at 520 nm, using toluene as the blank, and L-proline as a standard.

2.7. Carotenoids determination

By using Arnon (1949) method, Carotenoid contents in the leaves were estimated. Fresh tissues (0.1 g) were homogenized in 80% ice-cold acetone and centrifuged at 4000 × *g* for 10 min. Carotenoid contents were calculated by absorbance at 470 nm. The content of carotenoid was expressed as $mg.g^{-1}$ FW.

2.8. Statistical analysis

The statically analysis was performed using the GLM procedure of SAS. All experiments were designated in triplicated. The differences between the mean values of treatments were compared by Duncan's test at ($p < 0.05$).

3. Results and discussion

3.1. Nickel concentrations

In this study, we found a positive correlation between the concentration of Ni in the shoot of *A. inflatum* populations and the total concentration of Ni in the medium. Several previous studies confirm the

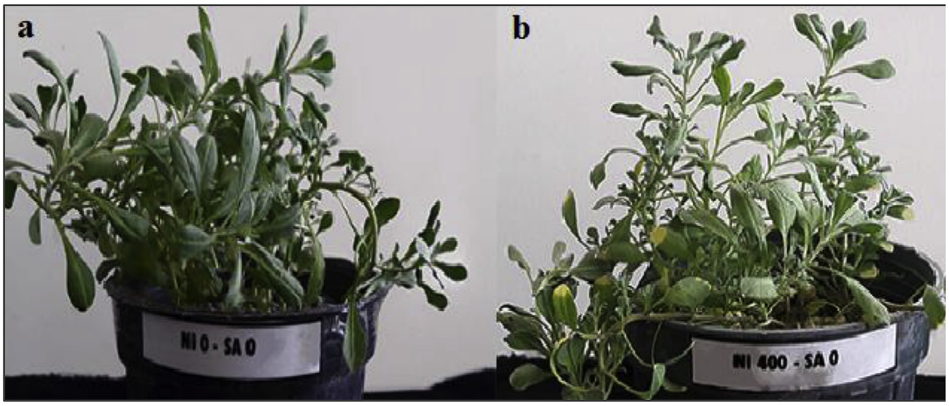

Fig. 2. The metallicolous population of *A. inflatum* (a) fed only with Hoagland's nutrient solution as the control, and (b) treated with 400 μM Ni.

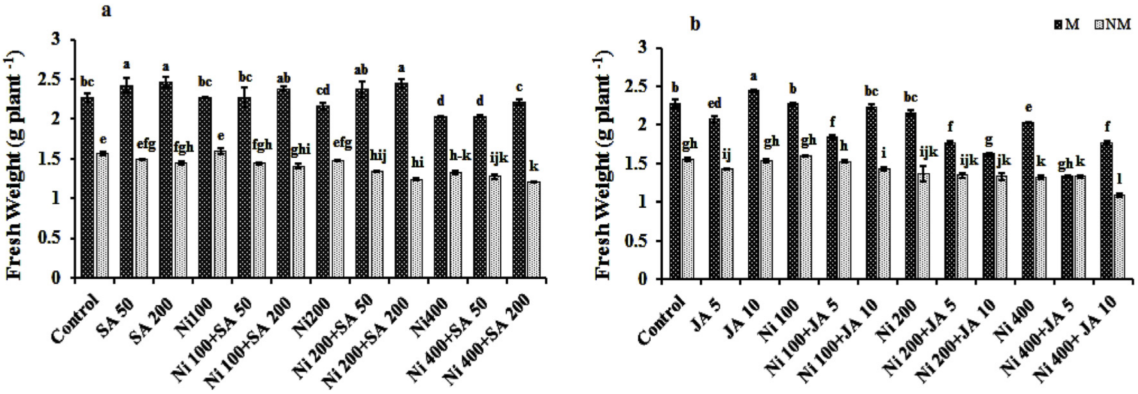

Fig. 3. The effect of SA (a) and JA (b) on FW in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (μM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

obtained results (Ghasemi et al., 2009; Ghaderian et al., 2015a,b). As shown in Fig. 1, Ni concentrations in shoots of both population (M and NM) remarkably increased with incrementing Ni exposure levels. For example, compared to control, Ni concentration in the shoot of both M and NM populations treated with 400 μM Ni increased 72 and 92 folds, respectively, compared to the control population. There was no significant difference between M and NM populations regarding Ni accumulation in plants treated with 400 μM Ni. It is confirmed that this plant species has a high ability to tolerate high concentrations of Ni.

The decrease in Ni accumulation was observed in plants treated with the interaction of JA and SA with Ni (Table 1). In M and NM populations treated with Ni 400 μM + SA 200 μM, Ni accumulation was reduced by 43.0% and 29.0% in M and NM populations, respectively, compared to plants treated with 400 μM Ni alone. Previous studies showed that exogenous SA caused the reduction in Al accumulation in *Cassia tora* and exogenous JA led to decrease in Cd concentration in *Solanum nigrum* (Yang et al., 2003; Yan et al., 2015). The results suggest that SA and JA, probably involved in the direction of the stress tolerance mechanisms of Ni, prevent the Ni accumulation in the root, transfer, and accumulation in the shoot.

3.2. Plant fresh weight

Reduction of plant growth due to Ni stress is the primary visible symptom that demonstrates the extent of toxicity induced. The growth variable examined in this study, including the FW of shoots, showed a significant decrease with an increase in Ni concentration by 10.6% and 20.6% in both M and NM plants, respectively. In spite of the highest growth reduction in plants treated with 400 μM Ni, there were low toxicity symptoms manifested in *A. inflatum* under Ni stress, demonstrating its great Ni tolerance (Fig. 2). Hence, in this study the highest FWs were observed in the M population treated with Ni 200 μM + SA 200 μM (Fig. 3 a) and, JA 10 μM (Fig. 3 b). The ameliorative effects of exogenous SA + JA on some growth parameters of *A. inflatum* under Ni stress (Ni 400 μM + SA 50 μM + JA 10 μM) was approved in this investigation (Table 1). There have been several reports that the biomass of *A. inflatum* decreased with increasing rate of Ni concentration in the medium (Ghasemi et al., 2009; Ghaderian et al., 2015a,b). This decrease in biomass depends on some physiological responses amongst plants such as Ni accumulation and, apoplastic Ni binding efficiency. Shoot apoplast acts as an important sink for excessive Ni, especially when large amounts of Ni are transported from the xylem to shoots (Deng et al., 2018). For example, *Leptoplax emarginata* as Ni hyperaccumulator possess an extremely high apoplast capacity to take up Ni (Redjala et al., 2010) and probably other similar elements, such as Fe by increasing Ni concentrations in the culture medium (taken up and replacement of Ni by the apoplast and replacement of Ni with other elements in apoplast) (Ghaderian et al., 2015a,b). High Ni nutrition of plants leads to the release of nutrients from apoplast and prevents absorption of other nutrient elements such as K, Fe, Cu, Mg, Mn and Ca in *Berkhya coddii* from roots (Robinson et al., 2003; Dalcorso et al., 2014). All these processes reduce growth parameters. The effect of exogenous SA on growth parameters appears to be dependent on the plant species, developmental stage, and SA concentrations (Rivas-San Vicente and Plasencia, 2011). In their study, Yan et al. (2013) showed that biomass was enhanced with exogenous application of methyl JA (0.1 μM) in *Capsicum frutescens* under Cd stress. Similarly, Ahmad et al. (2017) reported an increase in dry weight by 40.9% in faba bean treated with JA + Cd compared to Cd-treated plants. Also, Keramat et al. (2009) have reported that exogenous JA

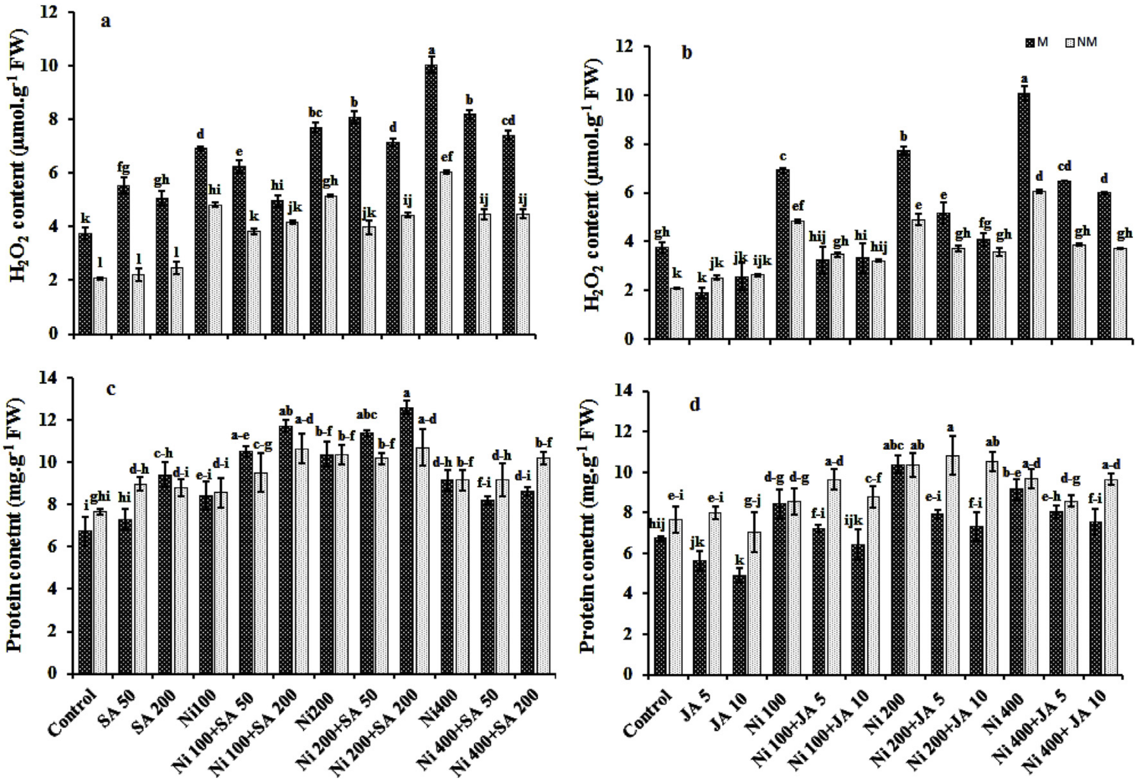

Fig. 4. The effect of SA (a) and JA (b) on H_2O_2 content and the effect of SA (c) and JA (d) on total soluble protein concentration in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (μM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

amended shoot biomass in soybean by improving total chlorophyll content under Cd stress. Therefore, the positive effects of SA and JA on growth improving *A. inflatum* populations could be related to changes in the hormonal status or by the enhancement of other physiological parameters such as photosynthesis, transpiration, and stomatal conductance. For instance, abscisic acid (ABA) regulates stomatal functioning, impaired pigment biosynthesis, and the concomitant enhancement of total antioxidant activity of shoots (Hayat et al., 2010; Rivas-San Vicente and Plasencia, 2011; Alam et al., 2014).

3.3. Hydrogen peroxide contents

The concentration of H_2O_2 as a potent ROS gradually increased with increasing Ni exposure level in both populations (Fig. 4 a, b). Results showed that at 400 μM treatment with Ni, the highest H_2O_2 content was generated in both populations (2.6 and 2.9-folds H_2O_2 production compared to the control in M and NM populations, respectively). Other works on *A. inflatum* approved these findings (Ghasemi et al., 2009; Ghaderian et al., 2015a,b). The existence of high Ni concentration could stimulate the NADPH oxidase enzyme in the plasma membrane, and produce H_2O_2 (Hao et al., 2006). Moreover, in chloroplasts and mitochondria, H_2O_2 was reported to generate from $O_2^{\cdot-}$ by SOD activity (Wang et al., 2009). The existence of SA and JA in lower concentration in the plants under high Ni stress confirmed the role of JA and SA in the plant as a defensive mechanism against oxidative damage. Consequently, at Ni 400 μM + SA 50 μM + JA 5 μM, generation of H_2O_2 was significantly reduced by 63.2% and 38.8% compared to plants-treated with Ni alone (400 μM) in M and NM populations, respectively (Fig. 5 a). According to these results, exogenous SA reduced the H_2O_2 content in *Brassica juncea* (Yusuf et al., 2012), *Eleusine coracana* (Kotapati et al., 2017), and *L. minor* (Lu et al., 2018) treated with Ni. Similarly, the application of exogenous JA in soybean (Sirhindi et al., 2015, 2016) treated with Ni was proved to reduce the generation and accumulation of H_2O_2 and other ROS content, further supporting our results. In the presence of JA the reduction of H_2O_2 may be due to: (i) increasing proline contents, which has the antioxidant feature and decline the ROS produced and (ii) elevated antioxidative system that could also minimize the generation of ROS (Shan and Liang, 2010; Sirhindi et al., 2016).

3.4. Soluble protein content

As shown in Fig. 4, a total protein in plants treated with Ni 200 μM, a total protein content showed an increase in both M and NM populations by 35.0% and 26.3% respectively, compared to control. However, by increasing Ni level to 400 μM in the medium, protein content was declined by 11.7% and 11.8% compared to plants treated with 200 μM Ni, in M and NM populations, respectively. Reduction of protein contents in plants treated with Ni can be associated with: (i) high production of ROS that could more degrade proteins (Sirhindi et al., 2016), (ii) interaction with proteins containing SH-group that inhibit enzymes activity by changes in protein conformation (Seregin and Kozhevnikova, 2006), (iii) elevation of protease activity which resulted in degradation of proteins (Palma et al., 2002; Sirhindi et al., 2016). The addition of SA (200 μM) to M population under Ni 200 μM and 400 μM (Fig. 4 c) increased protein content by 11.0% and 21.4% respectively, compared to respective control plants treated with Ni 200 μM and 400 μM only. Under Ni 200 μM + JA 5 μM treatment (Fig. 4 d), enhanced the protein concentration by 4.1% than Ni 200 μM in NM population. As well, in the M population treated with Ni 400 μM + SA 50 μM + JA 5 μM, total protein was incremented (Fig. 5 b). According to these results, SA and JA at lower concentration improve the toxic effect of Ni on protein content. In a study by Sirhindi et al. (2016), the protein content of soybean was enhanced by 22.6% under Ni stress while under the interaction of JA and Ni protein content was elevated by 58.4%.

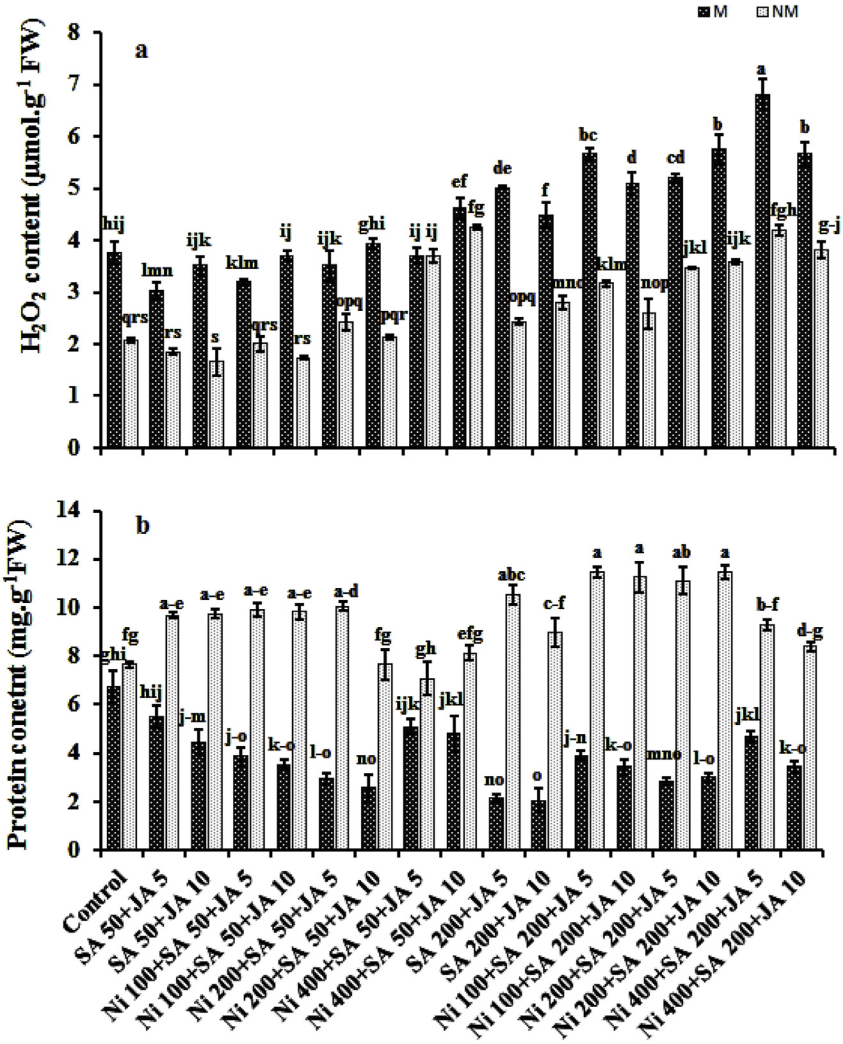

Fig. 5. The effect of exogenous SA + JA on (a) H_2O_2 content and (b) total soluble protein content in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (µM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

3.5. Enzymes activity

Exposure of both M and NM populations to Ni (Ni 200 µM and 400 µM) increased antioxidant enzymes activities (SOD, CAT, POD, and APX) compared to control (Fig. 6). Overall, the significant difference in antioxidant enzymes activity was recorded between M and NM populations. By enhancing Ni concentration in the medium, SOD and APX activity of M plants were incremented, compared to NM plants. The increase was also evident in CAT and APX activity of NM population. The activity of antioxidant enzymes may vary according to plant species, plant tissue, type of stress, and stress duration between plant species (Sreekanth et al., 2013). Gajewska et al. (2006) indicated that in the shoot of wheat exposed to Ni, the APX and POD activities increased, while SOD and CAT activities displayed a decrease. As mentioned above, a sub-lethal dose of Ni may directly bind with the thiol group of cysteine (Cys 32) in the active site of APX, which leads to inhibition of enzyme activity. Therefore, it could be the reason for blockage of APX activity in NM plants, as the enzyme is sensitive to thiol reagents (Chen and Asada, 1989; Seregin and Kozhevnikova, 2006).

Furthermore, SOD activity showed an increasing trend in both populations by adding SA to the medium of plants affected by Ni (400 µM) stress (Fig. 6 a). This increment in the M population was almost 2-fold higher than in NM population. In NM, a modest increase was observed, whereby an increase in Ni concentration (400 µM) resulted in SOD activity elevated by 28.1% compared to the control. In the M population treated with 400 µM Ni, SOD activity showed a decrease by 9.6% compared to population treated with 200 µM Ni. Ghasemi et al. (2009), found that in the presence of toxic concentrations of Ni, the activity of Fe-SOD decreases in the shoots, though the activity of Cu-SOD and Mn-SOD remains unchanged. Therefore, this remarkable decrease in Fe-SOD activity under toxic Ni concentrations was probably due to the Ni-induced Fe deficiency in the shoots. On the other hand, by application of SA, uptake of Fe^{2+} can be improved, and SA could also raise Fe-SOD activity in Ni stress conditions (Kotapati et al., 2017). The same results were acquired in maize exposed to Cd (Krantev et al., 2008), *Catharanthus roseus* under Ni stress (Idrees et al., 2013), and *Nymphaea tetragona* under Cd stress (Gu et al., 2018).

By increasing Ni level and treatments with SA and JA, activities of SOD, POD, and CAT were increased in both populations, albeit, APX activity was reduced (Table 2). In a study by Ahmad et al. (2017), with the application of JA, the activity of SOD, CAT, and AXP in faba bean was considerably enhanced in the presence of Cd. This result is also supported by a study of Sirhindi et al. (2016) and Lu et al. (2018). They demonstrated the activities of SOD, POD, CAT, and APX increase in Ni and Cd-treated soybean and *Lemna minor* by the application of JA and SA, respectively. The JA may stimulate other endogenous phytohormones that could directly or indirectly impart tolerance to plants through the low production of ROS (Kang et al., 2005). The SA, as a true

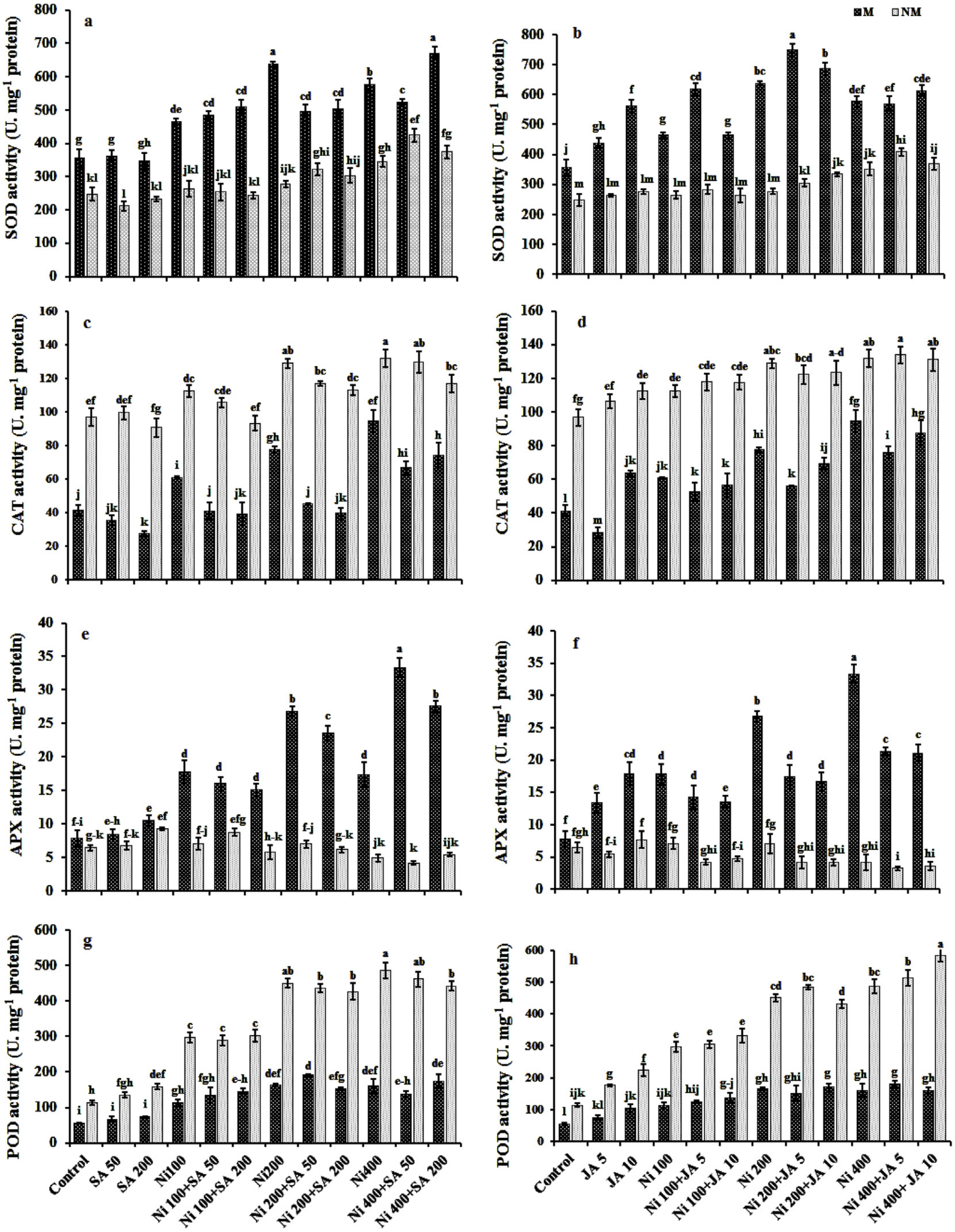

Fig. 6. The effect of SA and JA on (a, b) SOD activity, (c, d) CAT activity, (e, f) APX activity and (g, h) POD activity in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (μM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at p ≤ 0.05) is indicated with different letters according to the Duncan test.

plant hormone, ameliorates the defense response in plant immunity by coordination with the other hormones such as JA (Khan et al., 2015).

3.6. Proline contents

The proline accumulation in both M and NM populations considerably increased with increasing exposure to Ni. However, there is a significant difference between both populations (at $p < 0.05$) so that, whereby the proline content in NM populations was 1.8-fold higher compared to M populations. According to some features such as regulation of osmotic pressure, conservation of membrane integrity, stabilization of enzymes/proteins, and preservation of suitable NADP/NADPH ratios as well as the scavenger of free radicals, proline accumulation capacity have been reportedly considered as a good indication of the self-protection of plants growing under stressful conditions (Gajewska et al., 2006; Misra and Saxena, 2009; Yan et al., 2015).

In the present study, the application of SA and JA resulted in an increase in proline accumulation in shoots of both populations treated with high Ni concentration (Fig. 7 a, b). For example, in plants treated with 400 μM Ni, application of SA 200 μM and JA 10 μM elevated proline content in the shoots by 25.8% and 44.5% in M and NM populations, respectively, compared to the control plants (Table 1). Therefore, in *A. inflatum* treated with SA and JA, there is a strong correlation between increased cellular proline levels and capacity to

Table 2
Effect of exogenous application of SA + JA on enzymes activity criteria in two populations of *A. inflatum* treated by different Ni levels.

CAT activity (U. mg^{-1} protein)		APX activity (U. mg^{-1} protein)		POD activity (U. mg^{-1} protein)		SOD activity (U. mg^{-1} protein)		Treatments
NM	M	NM	M	NM	M	NM	M	
96.7 ± 5.18 ^{hij}	41.3 ± 3.29 ^{op}	6.46 ± 0.41 ^{klm}	7.81 ± 1.18 ^{kl}	119 ± 11.5^{s}	55.5 ± 1.60^{t}	247 ± 19.5^{t}	356 ± 26.3^{s}	Control
84.6 ± 2.08 ^{kl}	25.8 ± 5.08^{q}	4.47 ± 0.59^{m}	3.28 ± 0.70^{m}	211 ± 21.0 ^{pqr}	227 ± 2.20 $^{n-r}$	283 ± 10.3^{t}	469 ± 12.4 ^{op}	(SA 50 + JA 5) μM
112 ± 3.26 ^{def}	33.2 ± 2.94 ^{pq}	5.28 ± 0.73 ^{lm}	6.05 ± 1.31 ^{klm}	208 ± 18.3 ^{qr}	187 ± 3.50^{r}	270 ± 28.5^{t}	888 ± 11.9^{i}	(SA 50 + JA 10) μM
88.2 ± 1.60 ^{jkl}	31.3 ± 1.53 ^{pq}	5.61 ± 0.74 ^{lm}	9.24 ± 1.40 ^{jk}	229 ± 17.9 $^{n-r}$	248 ± 4.20 $^{m-q}$	393 ± 10.9 ^{qrs}	1053 ± 18.5^{g}	(Ni 100 + SA 50 + JA 5) μM
119 ± 1.14 ^{cde}	48.4 ± 5.98 ^{no}	6.47 ± 0.55 ^{klm}	12.2 ± 1.70^{j}	218 ± 14.0 $^{o-r}$	293 ± 5.50 ^{klm}	349 ± 22.4^{s}	991 ± 10.2^{h}	(Ni 100 + SA 50 + JA 10) μM
99.5 ± 2.96 ^{ghi}	33.6 ± 5.98 ^{pq}	4.19 ± 0.38^{m}	16.3 ± 1.80^{i}	295 ± 35.1 ^{klm}	382 ± 6.07 ^{gh}	412 ± 14.9 ^{qr}	1791 ± 21.0^{b}	(Ni 200 + SA 50 + JA 5) μM
125 ± 3.66 ^{bc}	56.0 ± 2.65 ^{mn}	4.51 ± 0.48 ^{kl}	21.8 ± 1.14 ^{gh}	272 ± 2.70 $^{k-o}$	478 ± 7.10 ^{cd}	424 ± 23.7 ^{pq}	1936 ± 21.4^{a}	(Ni 200 + SA 50 + JA 10) μM
147 ± 2.54^{a}	90.1 ± 0.82 ^{ijk}	5.84 ± 0.16 ^{lm}	32.5 ± 2.10 ^{cd}	323 ± 23.3 ^{ijk}	307 ± 8.09 ^{jkl}	663 ± 6.30^{k}	839 ± 32.9 ^{ij}	(Ni 400- SA 50 + JA 5) μM
121 ± 3.57 ^{bcd}	92.8 ± 4.95 ^{ijk}	4.28 ± 0.40^{m}	44.9 ± 1.70^{b}	355 ± 8.90 ^{hij}	384 ± 9.20 ^{gh}	571 ± 19.5 ^{lm}	1078 ± 41.1^{g}	(Ni 400 + SA 50 + JA 10) μM
107 ± 4.39 ^{fg}	78.6 ± 1.53^{l}	5.25 ± 0.86 ^{lm}	34.4 ± 2.50^{c}	264 ± 31.8 $^{l-p}$	453 ± 10.1 $^{c-f}$	186 ± 12.6^{u}	1311 ± 12.2^{f}	(SA 200 + JA 5) μM
107 ± 4.23 ^{fg}	48.9 ± 3.32 ^{no}	6.52 ± 0.45 ^{klm}	29.9 ± 0.80 ^{de}	274 ± 29.1 $^{k-n}$	415 ± 11.9 ^{efg}	230 ± 18.1 ^{tu}	1315 ± 7.30^{f}	(SA 200 + JA 10) μM
110 ± 3.52 ^{ef}	34.8 ± 2.80 ^{pq}	6.01 ± 0.61 ^{klm}	30.6 ± 1.20^{d}	296 ± 37.8 ^{klm}	455 ± 21.5 ^{def}	364 ± 22.0 ^{rs}	1585 ± 19.3^{d}	(Ni 100 + SA 200 + JA 5) μM
106 ± 5.77 ^{fgh}	37.5 ± 4.14^{p}	5.36 ± 1.08 ^{lk}	21.1 ± 1.40^{h}	288 ± 24.1 ^{klm}	458 ± 13.9 $^{c-f}$	352 ± 18.1^{s}	1340 ± 10.5^{f}	(Ni 100 + SA 200 + JA 10) μM
112 ± 2.67 ^{def}	65.3 ± 1.97^{m}	4.81 ± 0.32 ^{lm}	55.5 ± 1.80^{a}	375 ± 22.0 ^{ghi}	469 ± 14.2 ^{cde}	530 ± 26.9 ^{mn}	1884 ± 18.0^{a}	(Ni 200 + SA 200 + JA 5) μM
123 ± 2.31 ^{bc}	99.1 ± 2.59 ^{ghi}	3.92 ± 0.19^{l}	35.1 ± 0.60^{c}	359 ± 27.5 $^{g-j}$	617 ± 15.3^{b}	492 ± 11.2 ^{no}	1409 ± 13.9^{e}	(Ni 200 + SA 200 + JA 10) μM
130 ± 1.18^{b}	146 ± 4.56^{a}	3.85 ± 0.39^{m}	27.0 ± 1.60 ^{ef}	406 ± 16.5 ^{fgh}	508 ± 16.3^{c}	611 ± 14.2 ^{kl}	1603 ± 12.2^{d}	(Ni 400 + SA 200 + JA 5) μM
150 ± 5.40^{a}	150 ± 5.72^{a}	4.67 ± 0.66 ^{lm}	24.5 ± 1.40 ^{fg}	317 ± 3.80 ^{jkl}	687 ± 17.7^{a}	814 ± 9.30^{j}	1735 ± 25.7^{c}	(Ni 400 + SA 200 + JA 10) μM

The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at $p \leq 0.05$) is indicated with different letters according to the Duncan test.

survive the effects of high Ni stress. Similarly to our results, enhanced proline accumulation in response to Cd toxicity has been demonstrated in maize leaves, though the application of SA decreased the stimulatory effect of Cd on proline accumulation (Al-Mureish, 2014). Furthermore, in *Eleusine coracana* treated with SA, proline content decreased under Ni stress (Kotapati et al., 2017). Proline content was found enhanced in tomato treated with JA and Cd stress (Zhao et al., 2016). Another study reported that the pretreatment of soybean seeds with JA causes a minor increase of proline content in the Ni-treated seedlings relative to the control (Mir et al., 2018).

3.7. *Carotenoid*

Inside the membrane phase, carotenoids play a role as one of the most important compounds that control the amount of ROS and derived molecules. Furthermore, to their ancillary light-gathering roles, these pigments operate in a non-redox role to de-excite triplet chlorophyll or singlet oxygen (Noctor et al., 2018). *A. Inflatum* populations exposed to highest Ni levels exhibited a significant decrease in carotenoid content, 13.6% in M and 28.7% in NM, compared to control. The change in carotenoid contents under Ni stress has already been reported by Salehi Eskandari et al. (2017) in *Cleome foliolosa*. The interaction of Ni stress and hormonal treatment was also significant for carotenoid in two populations of *A. inflatum* ($p < 0.05$). Namely, exogenous SA or JA application ameliorated carotenoid contents in NM population under 400 μM Ni concentration (Fig. 7 c, d). In soybean treated with JA, the content of carotenoid increased under Cd stress (Sirhindi et al., 2015). Furthermore, SA application increased carotenoid concentrations in shoots of maize (Wang et al., 2009), and *C. roseus* under Ni stress (Idrees et al., 2013). However, plants treated with Ni 400 μM + SA 200 μM + JA 5 μM showed an increment trend, although the increase in NM population was more pronounced than for M population (Table 1). According to these results, the application of low SA and JA levels lead to an increase in carotenoid content which ameliorated the deleterious effects of Ni stress.

4. Conclusion

The present study and the accessible literature review in this paper led to the conclusion that the exogenous application of SA and JA had a significant positive effect on growth parameters under Ni stress by reducing uptake and accumulation of Ni, especially, in the shoots of NM population. According to the results of current research, the potential free-form of SA and JA were able to ameliorate Ni oxidative stress by triggering antioxidant defense mechanisms in both *A. inflatum* populations. These forms included the SOD, CAT, POD, APX activities, proline, and carotenoid contents. These concurrently did reduce H_2O_2 content to remarkable levels. However, the responses of both populations were different in enzymes activity. The approximate elevation in the total soluble protein contents in the presence of SA and JA in plants treated with Ni indicates the role of these phytohormones to Ni tolerance in both of *A. inflatum* populations. Acquired data suggest that SA and JA are important plant hormones modifying the resistance of *A. inflatum* to Ni and enhances the stress or/and increases the plant resistance depending on its concentration. Metallicolous *A. inflatum* plant species may be of special interest for the rehabilitation of heavy-metals degraded lands. However, the interactive action between heavy metal, SA and JA is still unclear and requires further research in this regard.

Conflicts of interest

Authors declare that they have no conflict of interest.

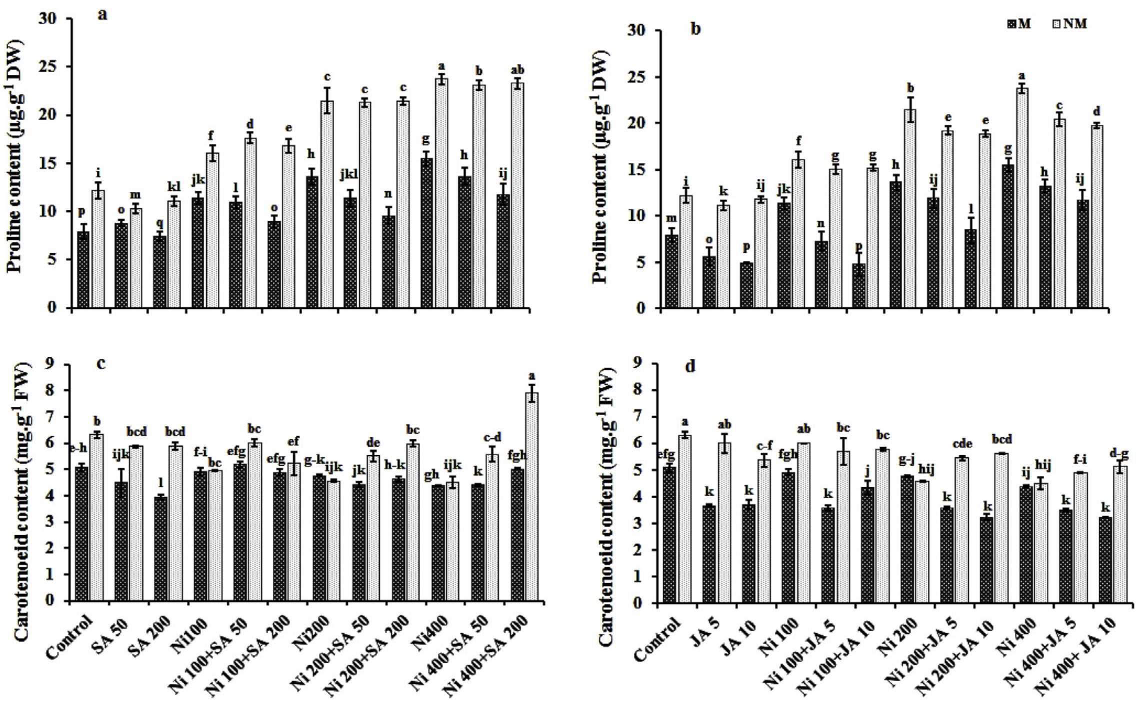

Fig. 7. The effect of SA (a) and JA (b) on proline content and the effect of SA (c) and JA (d) on carotenoid content in shoots of both populations of *A. inflatum* seedlings under Ni toxicity. All concentrations of Ni, SA, and JA were applied based on micromolar (μM). The values for each parameter are presented as mean ± SE; n = 3, while the significant difference between the two populations (at p ≤ 0.05) is indicated with different letters according to the Duncan test.

CRediT authorship contribution statement

Shiva Najafi kakavand: Formal analysis, Writing – original draft. **Naser Karimi:** Data curation, Supervision, Validation, Writing – review & editing. **Hamid-Reza Ghasempour:** Conceptualization, Writing – review & editing.

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References

Aebi, H., 1983. Catalase. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis. Academic Press, New York, pp. 273–286.

Ahmad, P., Alyemeni, M.N., Wijaya, L., Alam, P., Ahanger, M.A., Alamri, S.A., 2017. Jasmonic acid alleviates negative impacts of cadmium stress by modifying osmolytes and antioxidants in faba bean (Vicia faba L.). Arch. Agron Soil Sci. 63, 1889–1899. https://doi.org/10.1080/03650340.2017.1313406.

Ahmad, P., Nabi, G., Ashraf, M., 2011. Cadmium-induced oxidative damage in mustard [Brassica juncea (L.) Czern. & Coss.] plants can be alleviated by salicylic acid. South Afr. J. Bot. 77, 36–44. https://doi.org/10.1016/j.sajb.2010.05.003.

Al-Mureish, K., 2014. Salicylic acid-mediated alleviation of cadmium toxicity in maize leaves. J. Plant Sci. (Science Publ. Group) 2, 276. https://doi.org/10.11648/j.jps.20140206.13.

Alam, M.M., Nahar, K., Hasanuzzaman, M., Fujita, M., 2014. Exogenous jasmonic acid modulates the physiology, antioxidant defense, and glyoxalase systems in imparting drought stress tolerance in different Brassica species. Plant Biotechnol. Rep. 8, 279–293. https://doi.org/10.1007/s11816-014-0321-8.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplast, polyphenol oxidase in Beta vulgaris. Plant Physiol. 24, 1–15. https://doi.org/10.1104/pp.24.1.1.

Avalbaev, A., Yuldashev, R., Fedorova, K., Somov, K., Vysotskaya, L., Allagulova, C., Shakirova, F., 2016. Exogenous methyl jasmonate regulates cytokinin content by modulating cytokinin oxidase activity in wheat seedlings under salinity. J. Plant Physiol. 191, 101–110. https://doi.org/10.1016/j.jplph.2015.11.013.

Bankaji, I., Sleimi, N., López-Climent, M.F., Perez-Clemente, R.M., Gomez-Cadenas, A., 2014. Effects of combined abiotic stresses on growth, trace element accumulation, and phytohormone regulation in two halophytic species. J. Plant Growth Regul. 33, 632–643. https://doi.org/10.1007/s00344-014-9413-5.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Chance, B., Maehly, A.C., 1955. Assay of catalases and peroxidases. In: Methods in Enzymology, pp. 764–775. https://doi.org/10.1016/S0076-6879(55)02300-8.

Chen, C., Huang, D., Liu, J., 2009. Functions and toxicity of nickel in plants: recent advances and future prospects. Clean. - Soil, Air, Water 37, 304–313. https://doi.org/10.1002/clen.200800199.

Chen, G., Asada, K., 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol. 30, 987–998. https://doi.org/10.1093/oxfordjournals.pcp.a077844.

Dalcorso, G., Manara, A., Piasentin, S., Furini, A., 2014. Nutrient metal elements in plants. Metallomics. https://doi.org/10.1039/c4mt00173g.

Dar, T.A., Uddin, M., Khan, M.M.A., Hakeem, K.R., Jaleel, H., 2015. Jasmonates counter plant stress: a Review. Environ. Exp. Bot. 115, 49–57. https://doi.org/10.1016/j.envexpbot.2015.02.010.

Deng, T., van der Ent, A., Tang, Y., Sterckeman, T., Echevarria, G., Morel, J.-L., Qiu, R.-L., 2018. Nickel hyperaccumulation mechanisms: a review on the current state of knowledge. Plant Soil 423, 1–11. https://doi.org/10.1007/s11104-017-3539-8.

Faghih, S., Ghobadi, C., Zarei, A., 2017. Response of strawberry plant cv. 'camarosa' to salicylic acid and methyl jasmonate application under salt stress condition. J. Plant Growth Regul. 36, 651–659. https://doi.org/10.1007/s00344-017-9666-x.

Farhangi-Abriz, S., Ghassemi-Golezani, K., 2018. How can salicylic acid and jasmonic acid mitigate salt toxicity in soybean plants? Ecotoxicol. Environ. Saf. 147, 1010–1016. https://doi.org/10.1016/j.ecoenv.2017.09.070.

Gajewska, E., Skłodowska, M., Słaba, M., Mazur, J., 2006. Effect of nickel on antioxidative enzyme activities, proline and chlorophyll contents in wheat shoots. Biol. Plant. 50, 653–659. https://doi.org/10.1007/s10535-006-0102-5.

Ghaderian, S.M., Ghasemi, R., Hajihashemi, F., 2015a. Interaction of nickel and manganese in uptake, translocation, and accumulation by the nickel-hyperaccumulator plant, Alyssum bracteatum (Brassicaceae). Aust. J. Bot. 63, 47–55. https://doi.org/10.1071/BT14210.

Ghaderian, S.M., Ghasemi, R., Heidari, H., Vazirifar, S., 2015b. Effects of Ni on superoxide dismutase and glutathione reductase activities and thiol groups: a comparative study between Alyssum hyperaccumulator and non-accumulator species. Aust. J. Bot. 63, 65–71. https://doi.org/10.1071/BT14282.

Ghaderian, S.M., Mohtadi, A., Rahiminejad, M.R., Baker, A.J.M., 2007. Nickel and other metal uptake and accumulation by species of Alyssum (Brassicaceae) from the ultramafics of Iran. Environ. Pollut. 145, 293–298. https://doi.org/10.1016/j.envpol.2006.03.016.

Ghasemi, R., Ghaderian, S.M., 2009. Responses of two populations of an Iranian nickel-hyperaccumulating serpentine plant, Alyssum inflatum Nyar., to substrate Ca/Mg quotient and nickel. Environ. Exp. Bot. 67, 260–268. https://doi.org/10.1016/j.envexpbot.2009.06.016.

Ghasemi, R., Ghaderian, S.M., Krämer, U., 2009. Interference of nickel with copper and iron homeostasis contributes to metal toxicity symptoms in the nickel hyperaccumulator plant Alyssum inflatum. New Phytol. 184, 566–580. https://doi.org/10.

1111/j.1469-8137.2009.02993.x.

Ghasemi, R., Zare Chavoshi, Z., Boyd, R.S., Rajakaruna, N., 2014. A preliminary study of the role of nickel in enhancing flowering of the nickel hyperaccumulating plant Alyssum inflatum Nyár. (Brassicaceae). South Afr. J. Bot. 92, 47–52. https://doi.org/10.1016/j.sajb.2014.01.015.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol. 59, 309–314. https://doi.org/10.1104/pp.59.2.309.

Gu, C.S., Yang, Y.H., Shao, Y.F., Wu, K.W., Liu, Z.L., 2018. The effects of exogenous salicylic acid on alleviating cadmium toxicity in Nymphaea tetragona Georgi. South Afr. J. Bot. 114, 267–271. https://doi.org/10.1016/j.sajb.2017.11.012.

Hao, F., Wang, X., Chen, J., 2006. Involvement of plasma membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci. 170, 151–158. https://doi.org/10.1016/j.plantsci.2005.08.014.

Hayat, Q., Hayat, S., Irfan, M., Ahmad, A., 2010. Effect of exogenous salicylic acid under changing environment: a review. Environ. Exp. Bot. 68, 14–25. https://doi.org/10.1016/j.envexpbot.2009.08.005.

Horváth, E., Pál, M., Szalai, G., Páldi, E., Janda, T., 2007. Exogenous 4-hydroxybenzoic acid and salicylic acid modulate the effect of short-term drought and freezing stress on wheat plants. Biol. Plant. 51, 480–487. https://doi.org/10.1007/s10535-007-0101-1.

Idrees, M., Naeem, M., Aftab, T., Khan, M.M.A., Moinuddin, 2013. Salicylic acid restrains nickel toxicity, improves antioxidant defence system and enhances the production of anticancer alkaloids in Catharanthus roseus (L.). J. Hazard Mater. 252–253, 367–374. https://doi.org/10.1016/j.jhazmat.2013.03.005.

Kang, D.-J., Seo, Y.-J., Lee, J.-D., Ishii, R., Kim, K.U., Shin, D.H., Park, S.K., Jang, S.W., Lee, I.-J., 2005. Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. J. Agron. Crop Sci. 191, 273–282. https://doi.org/10.1111/j.1439-037X.2005.00153.x.

Kaya, A., Doganlar, Z.B., 2016. Exogenous jasmonic acid induces stress tolerance in tobacco (Nicotiana tabacum) exposed to imazapic. Ecotoxicol. Environ. Saf. 124, 470–479. https://doi.org/10.1016/j.ecoenv.2015.11.026.

Keramat, B., Kalantari, K.M., Arvin, M.J., 2009. Effects of methyl jasmonate in regulating cadmium-induced oxidative stress in soybean plant (Glycine max L .). J. Microbiol. 3, 240–244. https://doi.org/10.1080/01904161003728685.

Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. Front. Plant Sci. 6. https://doi.org/10.3389/fpls.2015.00462.

Kotapati, K.V., Palaka, B.K., Ampasala, D.R., 2017. Alleviation of nickel toxicity in finger millet (Eleusine coracana L.) germinating seedlings by exogenous application of salicylic acid and nitric oxide. Crops J 5, 240–250. https://doi.org/10.1016/j.cj.2016.09.002.

Krantev, A., Yordanova, R., Janda, T., Szalai, G., Popova, L., 2008. Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J. Plant Physiol. 165, 920–931. https://doi.org/10.1016/j.jplph.2006.11.014.

Lu, Q., Zhang, T., Zhang, W., Su, C., Yang, Y., Hu, D., Xu, Q., 2018. Alleviation of cadmium toxicity in Lemna minor by exogenous salicylic acid. Ecotoxicol. Environ. Saf. 147, 500–508. https://doi.org/10.1016/j.ecoenv.2017.09.015.

Mahmood, M., Bidabadi, S.S., Ghobadi, C., Gray, D.J., 2012. Effect of methyl jasmonate treatments on the alleviation of polyethylene glycol-mediated water stress in banana (Musa acuminata cv. 'Berangan', AAA) shoot tip cultures. Plant Growth Regul. 68, 161–169. https://doi.org/10.1007/s10725-012-9702-6.

Mir, M.A., Sirhindi, G., Alyemeni, M.N., Alam, P., Ahmad, P., 2018. Jasmonic acid improves growth performance of soybean under nickel toxicity by regulating nickel uptake, redox balance, and oxidative stress metabolism. J. Plant Growth Regul. 0, 1–15. https://doi.org/10.1007/s00344-018-9814-y.

Misra, N., Saxena, P., 2009. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. Plant Sci. 177, 181–189. https://doi.org/10.1016/j.plantsci.2009.05.007.

Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232.

Noctor, G., Reichheld, J.-P., Foyer, C.H., 2018. ROS-related redox regulation and signaling in plants. Semin. Cell Dev. Biol. 80, 3–12. https://doi.org/10.1016/j.semcdb.2017.07.013.

Palma, J.M., Sandalio, L.M., Javier Corpas, F., Romero-Puertas, M.C., McCarthy, I., del Río, L.A., 2002. Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. Plant Physiol. Biochem. 40, 521–530. https://doi.org/10.1016/S0981-9428(02)01404-3.

Qiu, Z., Guo, J., Zhu, A., Zhang, L., Zhang, M., 2014. Exogenous jasmonic acid can enhance tolerance of wheat seedlings to salt stress. Ecotoxicol. Environ. Saf. 104, 202–208. https://doi.org/10.1016/j.ecoenv.2014.03.014.

Redjala, T., Sterckeman, T., Skiker, S., Echevarria, G., 2010. Contribution of apoplast and symplast to short-term nickel uptake by maize and Leptoplax emarginata roots. Environ. Exp. Bot. 68, 99–106. https://doi.org/10.1016/j.envexpbot.2009.10.010.

Rivas-San Vicente, M., Plasencia, J., 2011. Salicylic acid beyond defense: its role in plant growth and development. J. Exp. Bot. 62, 1–18. https://doi.org/10.1093/jxb/err031.

Rizwan, M., Mostofa, M.G., Ahmad, M.Z., Imtiaz, M., Mehmood, S., Adeel, M., Dai, Z., Li, Z., Aziz, O., Zhang, Y., Tu, S., 2018. Nitric oxide induces rice tolerance to excessive nickel by regulating nickel uptake, reactive oxygen species detoxification, and defense-related gene expression. Chemosphere 191, 23–35. https://doi.org/10.1016/j.chemosphere.2017.09.068.

Robinson, B.H., Lombi, E., Zhao, F.J., McGrath, S.P., 2003. Uptake and distribution of nickel and other metals in the hyperaccumulator Berkheya coddii. New Phytol. 158, 279–285. https://doi.org/10.1046/j.1469-8137.2003.00743.x.

Salehi Eskandari, B., Ghaderian, S.M., Schat, H., 2017. The role of nickel (Ni) and drought in serpentine adaptation: contrasting effects of Ni on osmoprotectants and oxidative stress markers in the serpentine endemic, Cleome heratensis, and the related non-serpentinophyte, Cleome foliolosa. Plant Soil 417, 183–195. https://doi.org/10.1007/s11104-017-3250-9.

Seregin, I.V., Kozhevnikova, A.D., 2006. Physiological role of nickel and its toxic effects on higher plants. Russ. J. Plant Physiol. 53, 257–277. https://doi.org/10.1134/S1021443706020178.

Sergiev, I., Academy, B., Alexieva, V., Academy, B., Yanev, S., Academy, B., 2000. Effect of atrazine and spermine on free proline content and some antioxidants in pea (pisum sativum L .) plants. Biol. Physiol. des Plantes 53, 63–66.

Shan, C., Liang, Z., 2010. Jasmonic acid regulates ascorbate and glutathione metabolism in Agropyron cristatum leaves under water stress. Plant Sci. 178, 130–139. https://doi.org/10.1016/j.plantsci.2009.11.002.

Sirhindi, G., Mir, M.A., Abd-Allah, E.F., Ahmad, P., Gucel, S., 2016. Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in Glycine max under nickel toxicity. Front. Plant Sci. 7, 1–12. https://doi.org/10.3389/fpls.2016.00591.

Sirhindi, G., Mir, M.A., Sharma, P., Gill, S.S., Kaur, H., Mushtaq, R., 2015. Modulatory role of jasmonic acid on photosynthetic pigments, antioxidants and stress markers of Glycine max??L. under nickel stress. Physiol. Mol. Biol. Plants 21, 559–565. https://doi.org/10.1007/s12298-015-0320-4.

Sreekanth, T.V.M., Nagajyothi, P.C., Lee, K.D., Prasad, T.N.V.K.V., 2013. Occurrence, physiological responses, and toxicity of nickel in plants. Int. J. Environ. Sci. Technol. 10, 1129–1140. https://doi.org/10.1007/s13762-013-0245-9.

Van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362, 319–334. https://doi.org/10.1007/s11104-012-1287-3.

Vatansever, R., Ozyigit, I.I., Filiz, E., 2017. Essential and beneficial trace elements in plants, and their transport in roots: a review. Appl. Biochem. Biotechnol. 181, 464–482. https://doi.org/10.1007/s12010-016-2224-3.

Wang, H., Feng, T., Peng, X., Yan, M., Tang, X., 2009. Up-regulation of chloroplastic antioxidant capacity is involved in alleviation of nickel toxicity of Zea mays L. by exogenous salicylic acid. Ecotoxicol. Environ. Saf. 72, 1354–1362. https://doi.org/10.1016/j.ecoenv.2009.03.008.

Yadav, V., Arif, N., Singh, S., Srivastava, P.K., Sharma, S., Tripathi, D.K., Dubey, N., Chauhan, D.K., 2016. Exogenous mineral regulation under heavy metal stress: advances and prospects. Biochem. Pharmacol. Open Access 5. https://doi.org/10.4172/2167-0501.1000220.

Yamaguchi, T., Tomioka, R., Takenaka, C., 2017. Accumulation of cobalt and nickel in tissues of Clethra barbinervis in a metal dosing trial. Plant Soil 421, 273–283. https://doi.org/10.1007/s11104-017-3455-y.

Yan, Z., Chen, J., Li, X., 2013. Methyl jasmonate as a modulator of Cd toxicity in Capsicum frutescens var. fasciculatum seedlings. Ecotoxicol. Environ. Saf. 98, 203–209. https://doi.org/10.1016/j.ecoenv.2013.08.019.

Yan, Z., Zhang, W., Chen, J., Li, X., 2015. Methyl jasmonate alleviates cadmium toxicity in Solanum nigrum by regulating metal uptake and antioxidative capacity. Biol. Plant. 59, 373–381. https://doi.org/10.1007/s10535-015-0491-4.

Yang, Z.-M., Wang, J., Wang, S.-H., Xu, L.-L., 2003. Salicylic acid-induced aluminum tolerance by modulation of citrate efflux from roots of Cassia tora L. Planta 217, 168–174. https://doi.org/10.1007/s00425-003-0980-0.

Yusuf, M., Fariduddin, Q., Varshney, P., Ahmad, A., 2012. Salicylic acid minimizes nickel and/or salinity-induced toxicity in Indian mustard (Brassica juncea) through an improved antioxidant system. Environ. Sci. Pollut. Res. 19, 8–18. https://doi.org/10.1007/s11356-011-0531-3.

Zhao, S., Ma, Q., Xu, X., Li, G., Hao, L., 2016. Tomato jasmonic acid-deficient mutant spr2 seedling response to cadmium stress. J. Plant Growth Regul. 35, 603–610. https://doi.org/10.1007/s00344-015-9563-0.

Zhou, Z.S., Guo, K., Elbaz, A.A., Yang, Z.M., 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of Medicago sativa. Environ. Exp. Bot. 65, 27–34. https://doi.org/10.1016/j.envexpbot.2008.06.001.