Chitosan Application Improves Nickel Toxicity Tolerance in Soybean

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Abstract

Nickel (Ni) is an essential micronutrient for plant growth and development; however, at high levels, it is strongly phytotoxic. Chitosan is a natural biopolymer which is obtained from the deacetylation of chitin and has a high potential to improve plant growth under normal and stressful conditions. In this experiment, the role of different methods of chitosan application including seed treatment (0.25 and 0.5%), soil application (0.5 and 1%), and foliar application (100 and 200 ppm) was investigated on reducing Ni toxicity (0 and 200 mg kg^{-1} soil) in soybean. Ni stress significantly reduced the growth attributes such as shoot length (33%), aerial biomass (23%), leaf area (25%), and seed yield (78%) which were related to the reduction of stomatal conductance, chlorophyll value, and relative water content (RWC). Ni toxicity also induced oxidative stress measured as malondialdehyde (MDA) and increased antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX), and proline content. Nonetheless, exogenous application of chitosan decreased Ni toxicity and improved the growth attributes via lowering Ni uptake and oxidative damage (as MDA level), increasing the stomatal conductance, chlorophyll value, and RWC and more enhancing the activities of antioxidant enzymes and proline content. Among the different methods of chitosan application, the foliar application at 100 ppm was the most effective treatment. In this treatment, under Ni stress conditions, soybean growth traits such as shoot length, aerial biomass, leaf area, and seed yield were increased by 39%, 24%, 24%, and 3.1-folds, respectively, compared to non-application of chitosan. Chitosan foliar application (100 ppm) could be recommended as a beneficial method for improving Ni toxicity tolerance in soybean.

Keywords Chitosan · Growth · Heavy metals · Nickel toxicity · Oxidative stress · Soybean

1 Introduction

Heavy metal pollution has become a serious global agricultural problem. Nickel (Ni) is an essential metal element for plant growth and development at low concentrations (0.01–10 µg g^{-1} dry weight). It's involved in the activation of some enzymes especially urease which is required for nitrogen metabolism (Rizwan et al. 2018). Ni is released within the environment through human activities, such as mining, smelting, burning of fossil fuels, application of phosphatic fertilizers, sewage sludge, vehicle emissions, municipal and industrial waste, steel manufacturing, and cement industry (Shahzad et al. 2018; Hassan et al.

2019). Although at low concentrations, Ni is an essential micronutrient; however, it may cause phytotoxicity and could lead to harmful effects in plants such as induced oxidative stress, reduced cell division, altered enzyme activities, declined water and nutrient uptake, caused chlorosis and necrosis, decreased gas exchanges and pigments content, and reduced germination, growth, and yield (Ameen et al. 2019; Hassan et al. 2019).

Various practices are applied to reduce heavy metal toxicity on plant growth. The exogenous application of natural and synthetic chemicals is one of these methods, in which the use of natural chemicals is more appropriate. Chitosan is a natural polycationic polymer obtained after the deacetylation of chitin. Chitin is an important component of crustacean shells like crab, shrimp, lobster, and also insects, mollusks, fungi, etc. Chitosan could be a proper carrier for agrochemicals, due to its bioactivity, biocompatibility, biodegradability, high permeability, economical, nontoxicity, and excellent film-forming ability. Chitosan structure could be simply altered to better uptake and slow

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release of plant growth regulators, fertilizers, pesticides, herbicides, etc. (Mujtaba et al. 2020). Chitosan induces beneficial responses in plants under normal and stressful conditions. However, its effectiveness depends on the concentration, structure, method of application, plant species, and growth stage (Hidangmayum et al. 2019). Foliar application of chitosan decreased cadmium (Cd) toxicity in edible rape (*Brassica rapa* L.) through reducing Cd accumulation and malondialdehyde (MDA) level as well as amelioration of chlorophyll content, photosynthesis, and antioxidant systems (Zong et al. 2017). Chitosan soil application improved Cd toxicity tolerance in radish (*Raphanus sativus* L.) not only by chelating the Cd within the soil and reducing Cd bioavailability but also via enhancing chlorophyll, sugar, soluble protein, and free amino acid contents and relative water content (RWC) (Farouk et al. 2011). The solution of chitosan oligosaccharides induced chilling resistance in cucumber (*Cucumis sativus* L.) fruit by stimulation of antioxidant enzymes and HSP gene expression and suppressing membrane lipid oxidation (Ru et al. 2020). Pretreatment of apple seedling leaves with chitosan solution (especially 100 mg L^{-1}) increased cell membrane stability and antioxidant enzyme activities under drought conditions (Yang et al. 2009). Seed treatment with chitosan improved salinity tolerance in rice seedlings by lowered MDA content as well as increased proline level and the activities of catalase (CAT) and peroxidase (POX) enzymes (Gonzalez et al. 2015).

Soybean (*Glycine max* L.) as a grain legume is a major source of vegetable protein and oil. Its seed contains approximately 40% protein and 20% oil which are valuable for human and animal nutrition; however, soybean productivity is reducing due to environmental stresses such as Ni toxicity (Sirhindi et al. 2016). Although some studies have shown that chitosan application improved the tolerance to environmental stresses in some plant species, limited information is available about the effect of chitosan on reducing Ni toxicity in soybean. On the other hand, a high level of chitosan may not be effective because it can increase ABA level by hydrogen peroxide signaling leading to stomatal closure, a saturation of chitosan receptor molecules which are present in the cell membranes (Hidangmayum et al. 2019), significant reduction of the cytoplasm, and condensation of the chromatin as well as increasing proteases activities (El Hadrami et al. 2010) and affecting the root meristem, cellular polarity, and/or cyclin-dependent kinases leading to disruption of root development (Asgari-Targhi et al. 2018). Therefore, it was hypothesized that various concentrations and application methods of chitosan would have different effects on Ni stress tolerance in soybean. Hence, the present research was designed and carried out to investigate the impact of chitosan application on improving Ni toxicity tolerance in soybean.

2 Materials and Methods

2.1 Growth Conditions, Treatments, and Experimental Design

This pot experiment was carried out in the greenhouse (temperatures 20/30 °C, relative humidity 60% and photoperiod 12 h) at the Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University (35° 35′ N; 51° 28′ E; 1000 m altitude), Tehran, Iran, during spring and summer 2018. Soybean (*Glycine max* L.) seeds (cv. Williams) were obtained from Pakan Bazr Corporation, Isfahan, Iran. Seeds without visible defect, insect damage, or malformation were surface sterilized using 5% sodium hypochlorite solution for 5 min and then rinsed three times with sterile distilled water. The plastic pots (50 cm in diameter and height) were filled with 11.3-kg soil containing an equal mixture of peat, decomposed manure, and farm soil. The texture of the soil was loam with pH 7.15; EC 1.72 dS m^{-1}; organic carbon 2.69%, N 0.24%; and P, K, and Ni 19.17, 408.78, and 0.85 mg kg^{-1} soil, respectively.

For Ni stress treatment, the soil of pots was thoroughly mixed with the appropriate amount of Ni sulfate ($NiSO_4.6H_2O$) to supply 0 and 200 mg Ni kg^{-1} soil (Kamran et al. 2016; Helaoui et al. 2020). Chitosan powder with a deacetylation degree of 90% was dissolved in 1% acetic acid and diluted in distilled water with various concentrations. In this research, three methods of chitosan application were applied to different concentrations including the soil application of chitosan as a mix with soil (0.5 and 1% of soil weight), seed treatment with chitosan (0.25 and 0.5%) for 12 h (Guan et al. 2009), and foliar spray of chitosan (100 and 200 ppm) in three times at 7-day intervals from the 4-leaf stage (Yang et al. 2009). This experiment was conducted in a completely randomized design (CRD) with eight treatments and four replicates as follows: (i) control, no-application of Ni or chitosan; (ii) Ni stress, 200-mg kg^{-1} soil; (iii) Ni stress + soil application of chitosan (0.5% of soil weight); (iv) Ni stress + soil application of chitosan (1% of soil weight); (v) Ni stress + seed treatment with chitosan (0.25%); (vi) Ni stress + seed treatment with chitosan (0.5%); (vii) Ni stress + foliar application of chitosan (100 ppm); and (viii) Ni stress + foliar application of chitosan (200 ppm). A total of 15 seeds were sown at a depth of 4 cm in each plastic pot which was irrigated regularly using tap water. After thinning at the 2-leaf stage, six uniform seedlings were retained per pot, which was equivalent to 30 plants m^{-2}, which is the optimal density of soybean in our region.

2.2 Measurement of Biochemical Traits

At the early flowering stage, some biochemical and physiological parameters were measured. Lipid peroxidation was

estimated in terms of MDA content according to the method of Heath and Packer (1968). Fresh leaf samples (500 mg) were homogenized in 10 mL of 0.1% (w/v) trichloroacetic acid (TCA), and the homogenate solution was centrifuged at 10000 rpm for 10 min. Then, 1 mL of the supernatant was mixed with 4 mL of 0.5% (w/v) thiobarbituric acid containing 20% (w/v) TCA. The mixture was heated at 95 °C for 30 min, cooled rapidly in an ice bath, and then centrifuged at 10000 rpm for 10 min. The absorbance of the supernatant was recorded at 532 nm. The value for nonspecific absorption at 600 nm was subtracted. The MDA content was calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} fresh weight.

Proline content was determined according to the method described by Bates et al. (1973). Fresh leaf samples (500 mg) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid, and the homogenate solution was centrifuged at 10000 rpm for 10 min. Then, 2 mL of the supernatant was added to 2-mL acid ninhydrin reagent and 2-mL glacial acetic acid in a test tube and placed in a water bath at 100 °C for 1 h. The reaction was terminated in an ice bath. Then, 4 mL of toluene was added to the reaction mixture and mixed vigorously. The toluene layer was separated from the aqueous phase and its absorbance read at 520 nm. The proline content was determined using a standard curve of L-proline and expressed as μmol g^{-1} fresh weight.

For preparing the crude enzyme extract, fresh leaf samples (500 mg) were homogenized in 5 mL of potassium phosphate buffer (100 mM, pH 7.0), containing 1 mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP), with the addition of 2-mM ascorbate in the case of ascorbate peroxidase assay. The homogenate was centrifuged at 15000 rpm for 20 min at 4 °C and the supernatant was used for the following enzyme assays. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm following the method of Beauchamp and Fridovich (1971). Catalase (CAT, EC 1.11.1.6) activity was determined by monitoring the decomposition of H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}) at 240 nm using the procedure of Aebi (1984). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to Nakano and Asada (1981) by the decrease in absorbance of ascorbate (extinction coefficient 2.8 mM^{-1} cm^{-1}) at 290 nm.

2.3 Measurement of Physiological Traits

The chlorophyll value of the youngest fully expanded leaves was measured using a chlorophyll meter (Spad, CL-01, Hansatech Instruments Ltd. England). Stomatal conductance was measured on a sunny day between 10:00 and 11:00 h on the youngest fully expanded leaves using a portable leaf porometer (SC-1, Decagon Devices, USA). To determine the relative water content (RWC), 10 disks (1 cm in diameter) from the middle portion of the youngest fully expanded leaves were collected, immediately weighed to record the fresh weight (FW), and then, rehydrated in Petri dishes containing distilled water for 24 h under dim light and room temperature to get the turgid weight (TW), and subsequently the disks were oven-dried at 70 °C for 48 h to record the dry weight (DW). RWC was calculated as RWC (%) = (FW - DW) / (TW - DW) × 100.

2.4 Ni Content Assessment

The oven-dried samples were grounded into a fine powder. Ni concentration in the root, shoot, and seed was estimated after digesting the samples in HNO_3-$HClO_4$ (3:1, v/v) using an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan).

2.5 Measurement of Growth Parameters

At the early flowering stage, the leaf area of plants was calculated using a leaf area meter (CI-202, CID Bio-Science, USA). At the physiological maturity stage, shoot length and seed yield (13% moisture) were determined. Then, all the aboveground plant parts were oven-dried at 80 °C for 48 h to determine the aerial biomass.

2.6 Statistical Analysis

The data were analyzed statistically with one-way analysis of variance using SPSS 17 statistical software (SPSS Inc., Chicago, IL, USA), and means were statistically compared by Duncan's multiple range test (DMRT) at $p \leq 0.05$. Data are expressed as means ± standard error (SE) of four replicates for each treatment.

3 Results

3.1 MDA Content

The MDA content as a biomarker of membrane lipid peroxidation markedly increased during Ni stress by 77% as compared to non-stress conditions. Nevertheless, the application of chitosan diminished the harmful effect of Ni on membrane lipid peroxidation and as a result decreased the MDA content. Among the chitosan treatments, foliar application at 100 ppm was the most effective (Table 1).

Table 1 Effects of different methods of chitosan application on some biochemical and physiological traits of soybean under nickel (Ni) toxicity

Treatments	MDA (nmol g⁻¹ F.W.)	SOD (Unit mg⁻¹ pro.)	CAT (Unit mg⁻¹ pro.)	APX (Unit mg⁻¹ pro.)	Prolin (μmol g⁻¹ F.W.)	Stomatal conductance (mmol m⁻² s⁻¹)	Chlorophyll value (Spad unit)	RWC (%)
T0	32.75±0.22g	55.59±0.22f	23.89±0.33e	20.16±0.29e	11.05±0.24c	66.64±2.63a	49.76±1.66a	84.35±1.84a
T1	57.99±0.95a	63.98±0.28e	32.23±0.39d	26.52±0.35d	14.94±0.33b	43.34±0.85f	31.70±0.80d	52.03±0.33f
T2	54.89±0.91b	72.80±0.32d	36.15±0.44c	29.54±0.38c	16.02±0.41ab	51.91±1.35e	41.87±0.95c	66.72±0.62e
T3	49.41±0.44c	73.26±0.39cd	37.25±0.51bc	31.05±0.44bc	16.65±0.50ab	55.73±1.66de	44.57±1.12b	71.80±0.93d
T4	47.56±0.39d	75.24±0.43cd	37.93±0.58bc	32.49±0.55b	16.78±0.55ab	56.35±1.92de	45.98±1.27b	73.38±1.29d
T5	45.65±0.36e	75.95±0.44bc	38.35±0.65ab	32.20±0.49ab	17.35±0.62ab	59.30±2.26bc	46.16±1.40b	74.73±1.52cd
T6	42.71±0.29f	79.89±0.65a	40.16±0.88a	33.57±0.70a	18.66±0.81a	62.57±2.59ab	46.74±1.58b	80.13±1.74b
T7	46.32±0.38de	78.50±0.52ab	38.49±0.70ab	33.29±0.62ab	17.45±0.75ab	61.55±2.48bcd	46.68±1.47b	77.44±1.65bc

Means within a column followed by the same letter(s) are not significantly different at $P \leq 0.05$ level using DMRT. Values are the mean ± SE ($n = 4$)

T0 = control (non-use of Ni and chitosan); T1 = Ni toxicity (200 mg kg⁻¹ soil); T2 = Ni + seed treatment with chitosan (0.25%); T3 = Ni + seed treatment with chitosan (0.5%); T4 = Ni + soil application of chitosan (0.5%); T5 = Ni + soil application of chitosan (1%); T6 = Ni + foliar application of chitosan (100 ppm); T7 = Ni + foliar application of chitosan (200 ppm)

3.2 Antioxidant Enzyme Activities

Ni toxicity increased SOD, CAT, and APX activities by 15, 35, and 32%, respectively, compared with the control. Moreover, exogenously applied chitosan, especially foliar application at 100 ppm, further raised the activities of these antioxidant enzymes under Ni stress (Table 1).

3.3 Proline Content

Ni stress elevated the proline content of soybean leaves by 36% in comparison with the control. Furthermore, chitosan application, especially foliar application at 100 ppm, caused a further increase in proline content under Ni toxicity (Table 1).

3.4 Stomatal Conductance

Ni toxicity caused a significant reduction in the stomatal conductance of soybean leaves by 35% in comparison with the control. Nonetheless, the exogenous supplication of chitosan elevated the stomatal conductance under Ni stress conditions. Among the chitosan treatments, foliar application at 100 ppm was the most effective (Table 1).

3.5 Chlorophyll Value

Upon exposure to Ni stress, the chlorophyll value of soybean leaves decreased noticeably by 36% compared to the control. However, exogenously chitosan, especially foliar application at 100 ppm, improved the chlorophyll value under Ni toxicity (Table 1).

3.6 RWC

Ni toxicity caused a significant decrease in the RWC of soybean plants by 38% as compared with the control. Nevertheless, the exogenous application of chitosan enhanced the RWC under Ni stress. The highest improvement in the RWC was achieved by foliar application of chitosan at 100 ppm (Table 1).

3.7 Ni Content

Ni stress severely increased Ni accumulation in the roots, shoots, and seeds of soybean plants as compared with the control. Ni accumulation was greater in the roots than in the shoots and seeds. These increases in the roots, shoots, and seeds were about 96.6-, 43.0-, and 26.3-folds, respectively, over the control. However, the application of chitosan, especially foliar application at 100 ppm, significantly decreased the accumulation of Ni in all measured plant parts (Table 2).

Table 2 Effects of different methods of chitosan application on Ni content and growth attributes of soybean under nickel (Ni) toxicity

Treatments	Root Ni ($\mu g\,g^{-1}$ D.W.)	Shoot Ni ($\mu g\,g^{-1}$ D.W.)	Seed Ni ($\mu g\,g^{-1}$ D.W.)	Shoot length (cm)	Aerial biomass (g plant^{-1})	Leaf area (cm^2 plant^{-1})
T0	0.28±0.03e	0.12±0.01e	0.085±0.003e	64.46±1.13a	152.1±2.78a	1201.9±14.14a
T1	27.05±0.95a	5.16±0.27a	2.23±0.09a	43.43±0.40f	116.8±1.00f	907.5±3.87d
T2	23.35±0.89b	4.04±0.21b	1.87±0.08b	47.86±0.42e	130.9±1.21e	993.3±5.36cd
T3	22.66±0.74b	3.76±0.11b	1.79±0.06b	49.46±0.44de	132.3±1.53de	1013.8±7.11c
T4	21.70±0.69b	3.66±0.10bc	1.78±0.05b	51.45±0.47d	133.5±1.66de	1074.4±9.10bc
T5	18.81±0.52c	3.32±0.09cd	1.46±0.04c	56.44±0.49c	137.3±1.94cd	1083.8±10.94bc
T6	14.11±0.22d	3.17±0.07d	1.15±0.02d	60.39±1.11b	144.4±2.56b	1129.8±12.95ab
T7	16.22±0.38d	3.28±0.08cd	1.33±0.03cd	57.71±0.89c	139.1±2.42bc	1082.8±10.88bc

Means within a column followed by the same letter(s) are not significantly different at $P \leq 0.05$ level using DMRT. Values are the mean ± SE ($n = 4$)

T0 = Control (non-use of Ni and chitosan); T1 = Ni toxicity (200 mg kg^{-1} soil); T2 = Ni + seed treatment with chitosan (0.25%); T3 = Ni + seed treatment with chitosan (0.5%); T4 = Ni + soil application of chitosan (0.5%); T5 = Ni + soil application of chitosan (1%); T6 = Ni + foliar application of chitosan (100 ppm); T7 = Ni + foliar application of chitosan (200 ppm)
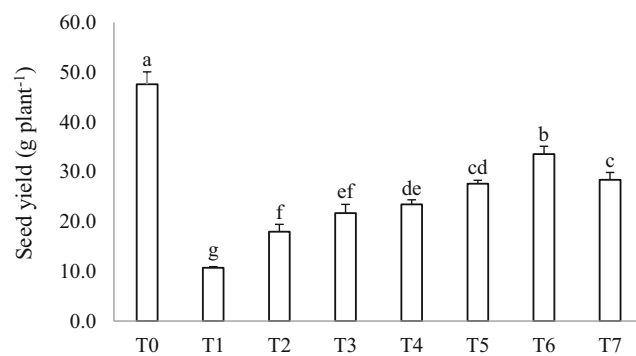

Fig. 1 Effects of different methods of chitosan application on seed yield of soybean under nickel (Ni) toxicity. Bars showing the same letters are not significantly different at $P \leq 0.05$ level using DMRT. Values are the mean ± SE ($n = 4$). Vertical bars show SE of the mean. T0 = control (non-use of Ni and chitosan); T1 = Ni toxicity (200 mg kg^{-1} soil); T2 = Ni + seed treatment with chitosan (0.25%); T3 = Ni + seed treatment with chitosan (0.5%); T4 = Ni + soil application of chitosan (0.5%); T5 = Ni + soil application of chitosan (1%); T6 = Ni + foliar application of chitosan (100 ppm); T7 = Ni + foliar application of chitosan (200 ppm)

3.8 Growth Parameters

Soybean growth attributes including shoot length, aerial biomass, leaf area, and seed yield noticeably reduced under Ni toxicity by 33, 23, 25, and 78%, respectively, compared with the control. Nonetheless, exogenously applied chitosan significantly improved the mentioned traits under Ni stress conditions. The highest improvement in terms of the growth parameters was recorded by foliar application of chitosan at 100 ppm (Table 2 and Fig. 1).

4 Discussion

A high level of Ni causes toxicity in plant cells by overproduction of reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide anion ($O_2\bullet^-$), hydroxyl radical ($\bullet OH$), and hydrogen peroxide (H_2O_2). At high concentrations, the ROS are extremely toxic and induce oxidative damage to proteins, DNA, and lipids and lead to membrane degradation and lipid peroxidation (Shahzad et al. 2018; Hassan et al. 2019). MDA a final product of membrane lipid peroxidation is a valid biomarker for measuring oxidative stress by heavy metal toxicity which increased noticeably in many plant species subjected to Ni toxicity (Siddiqui et al. 2011; Rizwan et al. 2018; Helaoui et al. 2020). In the current study also, the MDA level was raised markedly in Ni-treated soybean plants. However, exogenous application of chitosan (especially foliar application) lowered the MDA content under Ni stress, which is in accordance with the previous findings on edible rape (*Brassica rapa* L.) under Cd stress (Zong et al. 2017), maize under salt stress (Turk 2019), and safflower under drought stress (Mahdavi et al. 2011). To scavenging excessive ROS, plants have acquired an effective system of enzymatic

antioxidants (SOD, CAT, APX, POD, GR, etc.), as well as nonenzymatic antioxidants (ascorbic acid, glutathione, phenolics, tocopherols, etc.), to protect them against oxidative stress (Hassan et al. 2019). In the present experiment, antioxidant enzyme activities such as SOD, CAT, and APX were increased in response to Ni toxicity, which is in agreement with the previous studies on Indian mustard (*Brassica juncea* L.) (Zaid et al. 2019), cotton (Khaliq et al. 2016), and soybean (Sirhindi et al. 2016). In the current work although, these increased activities were not adequate to prevent oxidative damage, because they could not lower the MDA level under Ni stress conditions. However, exogenously applied chitosan effectively more elevated the activities of antioxidant enzymes as compared to the Ni alone and significantly reduced oxidative damage which was measured by MDA content. Similar reports of enhancing the activities of antioxidant enzymes by exogenous chitosan have been reported in different plant species exposed to Cd (Zong et al. 2017), salinity (Turk 2019), drought (Yang et al. 2009), and chilling stress (Ru et al. 2020). Enhanced activities of antioxidant enzymes by chitosan might be due to the upregulation of their gene expression (Ru et al. 2020). Moreover, the ability of chitosan in ROS scavenging might be attributed to the presence of abundant hydroxyl and amino groups in chitosan structure, which react with ROS (Hidangmayum et al. 2019). According to these findings, chitosan plays an important role in improving tolerance to Ni toxicity and reduces oxidative damage by direct scavenging ROS and also activating antioxidant enzymes.

In the present study, the proline level in soybean leaves was increased under Ni stress; moreover, exogenous application of chitosan (especially foliar application) more elevated the proline level under Ni toxicity. Enhanced accumulation of osmolytes such as proline in the plant cells has been reported as one of the defense mechanisms against Ni toxicity in various plants (Siddiqui et al. 2011; Rizwan et al. 2018; Jahan et al. 2020). Increased proline content under Ni toxicity was attributed to hydrolysis of proteins due to oxidative stress and also inhibition of proline degradation or enhancing de novo synthesis of proline (Kamran et al. 2016; Ameen et al. 2019). Proline, as an amino acid, increases tolerance to Ni toxicity by stabilizing cell membranes, buffering cellular redox potential, and scavenging ROS (Kamran et al. 2016). The positive role of exogenously applied chitosan on raising proline level under Ni toxicity in soybean which was observed in the current research and has also been reported in rice under salinity (Gonzalez et al. 2015) and maize under low-temperature stress (Guan et al. 2009). This might be due to the stimulation of enzymes involved in proline biosynthesis and/or inhibition of proline oxidase.

Stomatal conductance is one of the most effective factors in gas exchange, photosynthesis, transpiration, water uptake, and plant cooling. In this experiment, Ni toxicity significantly reduced the stomatal conductance of soybean leaves. Similarly,

several studies showed that Ni stress induced the closure of stomata in many plant species (Khaliq et al. 2016; Zaid et al. 2019; Jahan et al. 2020). This may be ascribed to a decrease in leaf area, leaf water content, and an increase in the ABA level (Ameen et al. 2019), as well as altering K^+ fluxes across the guard cell membranes (Hassan et al. 2019). In the present study, exogenously chitosan (especially foliar application) improved the stomatal conductance of soybean leaves under Ni stress conditions. This might be due to the amelioration of water status in plants. In conformity with these results, Zong et al. (2017) reported that Cd toxicity suppressed the stomatal conductance of edible rape plants while a foliar spray of chitosan significantly increased the stomatal conductance and promoted the photosynthesis.

In the current study, Ni toxicity markedly reduced the chlorophyll value of soybean leaves; on the other hand, the chitosan application ameliorated the chlorophyll value under Ni stress. The reduction in the chlorophyll content in different plant species under Ni stress has been reported by other researchers (Gajewska et al. 2006; Kumar et al. 2018; Helaoui et al. 2020). This might be due to the inhibition of α-aminolevulinic acid dehydratase (ALA-dehydratase) and protochlorophyllide reductase which are involved in the biosynthesis of chlorophyll, stimulation of chlorophyllase activity, Mg^{2+} and Fe^{2+} deficiency, peroxidation of chloroplast membrane, reduction of number and size of chloroplast and disorganization of ultra-structure, and reducing grana and thylakoid numbers (Sirhindi et al. 2016; Shahzad et al. 2018; Zaid et al. 2019). The beneficial function of chitosan on the chlorophyll content was attributed to stimulation of the expression of genes which are involved in the chlorophyll biosynthetic pathway, suppression of the transcript level of chlorophyllase which is involved in the catabolic pathway of chlorophyll, (Mukhtar Ahmed et al. 2020), enhancing endogenous levels of cytokinins which stimulate chlorophyll synthesis, much availability of amino compounds released from chitosan, and increasing nitrogen and potassium content in plant shoots which cause increasing the number of chloroplasts per cell (Farouk et al. 2011; Hidangmayum et al. 2019). In accordance with the present findings, exogenous application of chitosan enhanced the chlorophyll content in edible rape under Cd stress (Zong et al. 2017), cowpea under water stress (Farouk and Ramadan Amany 2012), and fenugreek (*Trigonella foenum-graecum* L.) under salinity stress (Mosapour Yahyaabadi et al. 2016).

RWC is an important indicator of water status in plants which is related to water uptake from the roots and transpiration rate from the leaves. In the present experiment, Ni toxicity significantly reduced the RWC of soybean leaves. In agreement with this result, Ni treatment decreased the RWC of Indian mustard (Zaid et al. 2019), tomato (Jahan et al. 2020), and wheat (Gajewska et al. 2006). This might be due to disturbed root growth, transpiration, stomatal movement, and

root hydraulic conductivity (Hassan et al. 2019). Nevertheless, in the current research, exogenous application of chitosan (especially foliar application) through increasing the stomatal conductance and proline accumulation improved the water relations in terms of the RWC of soybean plants under Ni stress. In accordance with this result, Mosapour Yahyaabadi et al. (2016) reported that seed treatment with chitosan improved the RWC of fenugreek plants under salt stress conditions. Similarly, Farouk et al. (2011) found that in radish plants subjected to Cd stress, exogenous application of chitosan improved the water status of plants via increasing the root growth (length and thickness), the diameter of the vascular cylinder, and also dimension of metaxylem vessels.

In the present work, when soybean plants were subjected to Ni, the accumulation of Ni in different plant parts increased so that the concentration of Ni in the roots was higher than in the shoots and seeds. It showed that most of the absorbed Ni remained in the roots and a small part of it was transported to the aerial parts. This retention was ascribed to Ni accumulation in the xylem vessel holes, cell walls, and vacuoles of cortical cells of the roots (Seregin and Kozhevnikova 2006). This result is in accordance with the previous reports on different plant species (Khaliq et al. 2016; Rizwan et al. 2017; Helaoui et al. 2020). Nevertheless, in the current experiment, exogenously applied chitosan (especially foliar application) significantly reduced Ni accumulation in the roots, shoots, and seeds of soybean plants. In line with the results of this study, chitosan treatment reduced the accumulation of Cd in radish (Farouk et al. 2011) and edible rape (Zong et al. 2017). Based on these results, one of the mechanisms for improving the tolerance to Ni toxicity by chitosan in soybean is to reduce the absorption and transfer of Ni.

In the current study, Ni toxicity through oxidative damage, reduction of stomatal conductance, chlorophyll value, and also RWC significantly reduced the growth attributes of soybean plants including shoot length, aerial biomass, leaf area, and seed yield. These results are in agreement with the previous findings on alfalfa (Helaoui et al. 2020), Indian mustard (Zaid et al. 2019), barley (Kumar et al. 2018), rice (Rizwan et al. 2017), and cotton (Khaliq et al. 2016). High levels of Ni decrease plant growth and development through induced oxidative stress, disturbance in water and minerals uptake, gas exchange, proteins, and lipid synthesis, cell division, and enzyme activities (Kumar et al. 2018; Hassan et al. 2019). Nevertheless, in the present work, exogenous application of chitosan (especially foliar application at 100 ppm), via reduction of Ni uptake and translocation, enhancing antioxidant enzyme activities, proline content, stomatal conductance, chlorophyll value, and RWC, ameliorated all measured growth attributes of soybean under Ni stress conditions. Similarly, the positive effects of exogenously applied chitosan on the growth traits of different plant species have been reported under Cd (Farouk et al. 2011), drought (Mahdavi et al.

2011; Farouk and Ramadan Amany 2012), salinity (Gonzalez et al. 2015; Turk 2019), and low-temperature stress (Guan et al. 2009). The beneficial role of chitosan on plant growth and development may be attributed to stimulation of a signal for the synthesis of plant growth regulators such as gibberellins and auxin, enhancing photosynthesis, glycolysis, and water and nutrient uptake, as well as regulation of nitrogen and carbon metabolisms (Farouk et al. 2011; Turk 2019; Mukhtar Ahmed et al. 2020).

It seems that in the foliar application of chitosan, its absorption and translocation to all plant parts was better. It is possible that the soil application of chitosan has caused part of it to be fixed, insoluble, or bacterially degraded. Moreover, soil-applied chitosan is often effective in controlling soil pathogens in many plant species. Chitosan seed treatment also has the greatest effect on the germination and seedling growth stages of the plant rather than other stages.

5 Conclusions

It could be concluded that nickel stress reduced the growth and yield of soybean plants by induced oxidative damage, decreased the stomatal conductance, chlorophyll value, and relative water content. Nonetheless, exogenous application of chitosan through reduction of nickel uptake and translocation, enhancing antioxidant enzyme activities, proline and chlorophyll values as well as ameliorating the gas exchanges and water status, improved the nickel toxicity tolerance of soybean. Among the seed treatment, soil and foliar application of chitosan, the foliar application at 100 ppm was the most effective treatment. Based on these findings, chitosan foliar application (100 ppm) could be suggested as a useful technique in alleviating the nickel toxicity in soybean.

Declarations

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