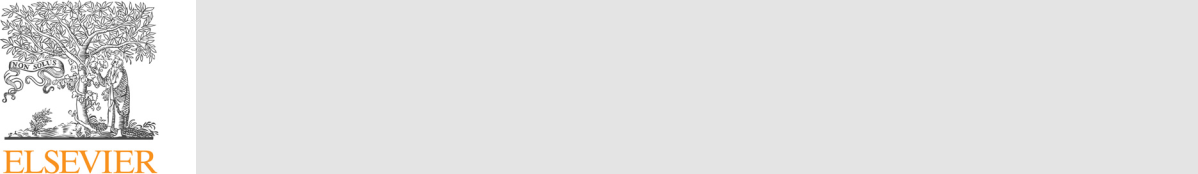
[Scientia Horticulturae 272 (2020) 109537](https://doi.org/10.1016/j.scienta.2020.109537)



Contents lists available at [ScienceDirect](http://www.sciencedirect.com/science/journal/03044238)



Scientia Horticulturae

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| Investigating the enzymatic and non-enzymatic antioxidant defense by | [T](http://crossmark.crossref.org/dialog/?doi=10.1016/j.scienta.2020.109537&domain=pdf) |  |
| applying iron oxide nanoparticles in Dracocephalum moldavica L. plant under |  |
|  |  |
| salinity stress |  |  |



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ARTICLE INFO

Keywords:

Antioxidative enzymes

Moldavian balm

Plant growth characteristics

Salt stress

Total phenolic content

ABSTRACT

The eﬀect of iron oxide nanoparticles (NPs), as a salinity stress modifier, was investigated on plant growth and antioxidant systems in Dracocephalum moldavica L. The results showed that the salinity causes a decrease in leaf area, length and fresh and dry weight of the shoot and root. However, foliar application of iron oxide NPs in the concentration of 60 ppm increased all aforementioned traits. Nevertheless, the interaction between iron oxide NPs and salinity was not significant on the length and weight (dry and fresh) of the shoot and root. Moreover, iron oxide NPs were not eﬃcacious on modifying the eﬀect of highest salinity level on the studied growth characteristics except for leaf area. Spraying the iron oxide NPs significantly increased the leaf area in the plants under salt stress conditions. Total phenolic, flavonoid and anthocyanin content, as well as the activity of guaiacol peroxidase, ascorbate peroxidase, catalase and glutathione reductase enzymes were enhanced in the shoot and root of the plants treated with 100 mM of NaC1 solution. On the other hand, spraying the iron oxide NPs on the plants treated with salinity stress led to an increase in enzymes activities compared to control ones. The results revealed that using iron nano fertilizers improves the antioxidant defense in Dracocephalum moldavica L. under salinity stress.

1. Introduction

Salinity, a combination of two osmotic and ionic stresses, is one of the most important environmental factors that results a decrease in plant growth and functions ([Yilmaz, 2007](#page1)). Additionally, these two stresses cause a second stress called “oxidative stress” ([Orcutt and](#page1) [Nilsen, 2000](#page1)). Reactive oxygen species (ROSs) produced during oxi-dative stress react with lipids, proteins, nucleic acids and cell enzymes and induce planned cell death pathway, ultimately leading to plant damage ([Gill and Tuteja, 2010](#page1)). Under natural conditions, there is a balance between the amount of ROSs production and scavenging. However, under severe environmental stresses, the balance is disturbed and the oxidative stress is produced in plants cells accordingly ([Hussain](#page1) [et al., 2016](#page1)).

Phenolic compounds can act as scavengers of oxygen free radicals or other ROSs ([Myung-Min et al., 2009](#page1)). According to the vital role of



phenolic compounds in scavenging the free radicals, extinguishing singlet oxygen, decomposition of peroxide and decreasing or inhibiting auto-oxidation of lipids, these compounds are considered as anti-oxidants, necessary for protecting plant cells against the proliferation and progression of oxidative stress ([Ksouri et al., 2007](#page1)). Phenolic compounds are soluble cell substances which modify the conditions during abiotic stress. In addition, accumulation of phenolic compounds in salinity tolerant plants is considered as a mechanism for inhibiting the ROS activity and protecting the cell membrane from salt stress damage ([Petridis et al., 2012](#page1); [Petropoulos et al., 2017](#page1)).

Anthocyanin is another antioxidant compound which not only re-moves free radicals, but also prevents their production in plants under salt stress conditions. It has been proven that plants which are able to produce and accumulate anthocyanin in epidermal layers, decrease oxidative stress eﬃciently ([Mittler et al., 2004](#page1); [Hare and Cress, 2007](#page1)). It seems that under water limited or salinity conditions, anthocyanin is

Abbreviations: Fe2O3 NPs, iron oxide nanoparticles; LAi, leaf area index; SL, shoot length; RL, root length; SFW, shoot fresh weight; RFW, root fresh weight; SDW, shoot dry weight; RDW, root dry weight; TEM, transmission electron microscopy; SEM, Scanning electron microscope; CAT, catalase; APX, ascorbate peroxidase; GPX, guaiacol peroxidase; GR, glutathione reductase; AsA, ascorbic acid; ROSs, reactive oxygen species; H2O2, Hydrogen peroxide; PAR, photosynthetically active radiation

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<https://doi.org/10.1016/j.scienta.2020.109537>

Received 19 June 2019; Received in revised form 19 May 2020; Accepted 3 June 2020

Available online 02 July 2020

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also able to act as an osmotic compatible solute ([Hughes et al., 2013](#page1)). Within Mehler pathway and glutathione-ascorbate cycle, the en-zyme ascorbate peroxidase (APX) plays a significant role in scavenging of H2O2 ([Sarker and Oba, 2018](#page1)). In glutathione-ascorbate cycle, as-corbate is oxidized into mono-hydro ascorbate via APX enzyme activity which is necessary for continuing ascorbate production process. For this purpose, mono dehydrated ascorbate reductase (MDHAR), dehydrated ascorbate reductase (DHAR), and glutathione reductase (GR) enzymes are activated and by using NADPH and glutathione the ascorbate is revived. GR plays a considerable role in plants’ adaption to oxidative stress. This enzyme is responsible for converting oxidized glutathione (GSSG) to reduced glutathione (GSH) maintaining the high ratio of GSH/GSSG. Hence, increasing GR due to re-reduction of oxidized glu-

tathione is very important ([Rao and Reddy, 2008](#page1)).

Peroxidases decompose H2O2 via oxidizing substrates including phenolic compounds or other antioxidants such as ascorbate and glu-tathione ([Parida and Das, 2005](#page1); [Pandey et al., 2017](#page1)). One of the im-portant ROS scavenging complexes is ascorbate peroxidase that has an important role in improving the tolerance of plants to saline-alkali conditions. It detoxifies H2O2 in diﬀerent compartments of the cell, resulting AsA homeostasis and ROS messenger network equilibrating ([Diaz-Vivancos et al., 2013](#page1)). Catalase (CAT) plays a role in decom-posing H2O2 produced during photorespiration in peroxisome or H2O2 produced during beta-oxidation of fatty acids in glyoxysomes without energy; however, it is only activated in high concentrations of H2O2. An increase in CAT activity is an adaptive response to overcome the da-mages resulting from H2O2 which is produced during cell metabolism ([Xie et al., 2019](#page1)).

On the other hand, salt stress makes change in metabolism of plants causing reduced plants growth, by reducing water absorption, creating imbalance in the absorption of nutrients and the toxic eﬀects of certain ions ([Hasni et al., 2009](#page1)). According to [Rui et al. (2009)](#page1) and [Memon](#page1) [et al. (2010)](#page1), salinity reduces the leaf area and the amount of photo-synthesis, both reducing plant growth and development.

Furthermore, the relations between salinity and micronutrient ele-ments are complicated. Salinity may cause increase or decrease the concentration of micronutrient elements in plant shoot, or it may have no eﬀect on their concentration. Although iron appears at high con-centrations in agricultural soils, its absorption is aﬀected by exogenous or endogenous factors in soil and plant resulting the lack of iron in diﬀerent tissues of plants. Most soils in arid and semi-arid areas are saline, thus, lack of iron is mostly observed in these areas. Foliar nu-trition is one of the eﬀective methods to satisfy plants’ nutritional needs to the elements ([Alshaal and El-Ramady, 2017](#page1)). The role of iron ele-ment in fixing nitrogen and activity of certain enzymes such as catalase, peroxidase and cytochrome oxidase has been investigated ([Tripathi](#page1) [et al., 2018](#page1)). Iron-sulfur proteins play a role in metabolism processes

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such as photosynthesis, reducing sulfate to sulfite, respiration, and fixing N2. Iron is eﬀective in making electron carriers in both photo-systems I and II ([Roncel et al., 2016](#page1)).

Nanotechnology, as an interdisciplinary technology and a pioneer in solving most of the scientific issues, has well-proven itself in agriculture science and the related industries. Nanotechnology has a wide range of applications in agriculture such as increasing crops productivity, re-ducing consumption of fertilizers and pesticides along with helping in conserving the natural resources on earth ([Rui et al., 2016](#page1)). Researchers believe that due to the small size and high solubility of iron NPs, they are absorbed faster by the plant that satisfy the lack of nutrition in plants. Therefore, these substances lead to optimal conditions for plant growth and prevent stress condition in plants and aﬀect secondary metabolite production positively ([Moradi et al., 2018](#page1)). Applying iron nanoparticles (NPs) can be an eﬀective step to reach sustainable agri-culture due to improving plant mineral nutrition and decreasing con-sumption of conventional fertilizers ([Rui et al., 2016](#page1); [Kopittke et al.,](#page1) [2019](#page1)). In previous studies, iron oxide NPs increased the antioxidant enzymes activity in wheat plants under salinity stress ([Babaei et al.,](#page1) [2017](#page1)), and improved antioxidant activity ([Rizwan et al., 2018](#page1)). Copper, silver, and gold NPs enhanced the accumulation of protein content, phenolics and flavonoids of Prunella vulgaris and Momordica charantia ([Fazal et al., 2016](#page1); [Chung et al., 2018](#page1)).

Moldavian balm, scientifically called Dracocephalum moldavica L. is an herbaceous and annual plant from Lamiaceae native to central Asia. The plant has been domesticated in eastern and central Europe ([Dastmalchi et al., 2007](#page1)). Moldavian balm essence has antimicrobial and antibacterial behavior and heals wounds and injuries. The essence of plant is widely used in pharmacology, cosmetics, nutritional, and perfume industry ([Nasrabadi et al., 2007](#page1)). Considering the importance and necessity of medicinal plants cultivation development and presence of limiting factors especially salinity in the Azarbaijan region and ac-cording to the available reports on the positive eﬀects of NPs applica-tion, this study aimed to investigate the eﬀect of Fe2O3 NPs on growth and antioxidants properties in the Moldavian balm plant in salinity conditions.

2. Materials and methods

2.1. Preparation of Fe2O3 nanoparticles

Fe2O3 NPs with 20 nm average particle size and 99.9 % purity were purchased from Iranians Nanomaterials Pioneers Company (Mashhad, Iran). The shape and size were determined by scanning electron mi-croscope (SEM) and transmission electron microscope (TEM) in Urmia University ([Fig. 1](#page1)). According to the information provided by the manufacturer, majority of the NPs had spherical morphology and the

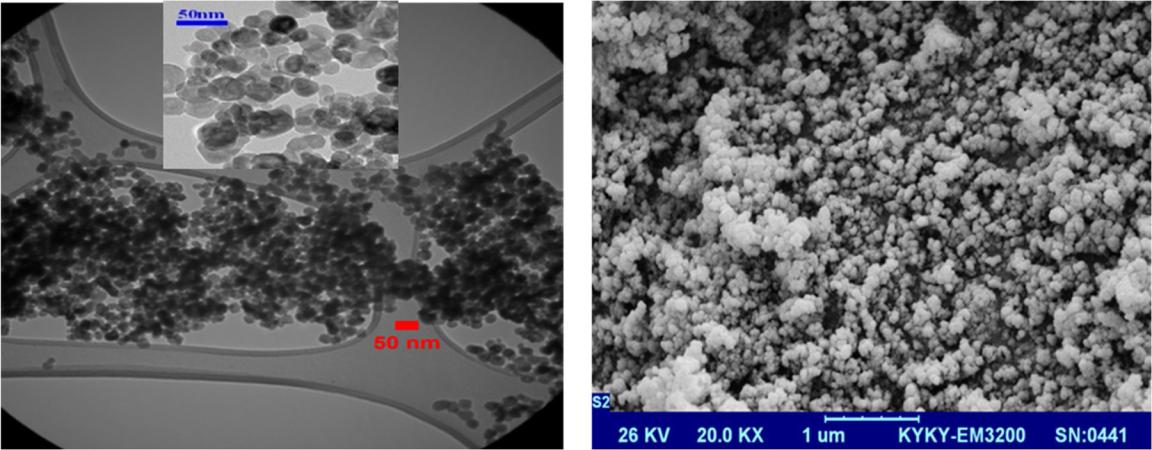


Fig. 1. Scanning electron microscope (SEM, Left) and transmission electron microscopy, (TEM, Right) images of Fe2O3 NPs. The shape of most NPs is spherical.

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diameter size ranged from 20–40 nm.

2.2. Growth conditions of Dracocephalum moldavica L. plants

In this research, the eﬀect of three levels of salinity (0, 50, and 100 mM NaC1) and iron oxide NPs in four levels (0, 30, 60, and 90 ppm) were investigated on Moldavian balm plant in a completely randomized design (CRD) with three replications. This experiment was carried out in the research greenhouse of Urmia University with tem-perature of 22−28 °C, PAR light intensity of 600 μmol m–2 s–1, a pho-toperiod of 16−8 h day/night and relative humidity of 45–60 %.

Seeds of Dracocephalum moldavica L. purchased from Pakanbazr Company (Isfahan, Iran) were planted in 10 L of perlite and sandy bed with 5: 1 ratio at 3 cm depth of soil. Ten seeds per pot were planted and in two true leaf stage, extra plants were removed and 5 plants were kept in each pot (three samples and replications). In order to maintain soil moisture near to field capacity, the irrigation of pots was carried out daily. The total plant growth time from planting to harvest was 7 weeks. From two true leaf stage, the pots were once watered with half strength Hoagland's solution and once again with distilled water. The salinity treatments include 0, 50 and 100 mM NaCl that were imposed to plants in the 8-leaf stage, applied daily (in combination with half-strength Hoagland solution) and continued for 20 days ([Gohari et al.,](#page1) [2020](#page1)). To prevent osmotic shock, salinity treatments were enhanced progressively by 25 mM NaCl every three days until the designated concentration was reached ([Chen et al., 2018](#page1)).

Iron oxide NPs with average diameter of 20–40 nm were used. The images of NPs powder samples taken by the scanning electron micro-scope (SEM) and transmission electron microscope (TEM) are shown in [Fig. 1](#page1). These NPs were immersed in the deionized water for 30 min by ultrasonic homogenizer (Cole-Parmer, 750-watt ultrasonic processor, 115 VAC) just before using to prevent aggregation. Then, the pH of solution was reduced with 1 mM HCl to dissolve all NPs ([Shi et al.,](#page1) [2000](#page1); [Tripathi et al., 2016](#page1)). Foliar application of iron oxide NPs treatments (50 mL) was performed at four levels (0, 30, 60, and 90 ppm) once a day at a certain time for a period of 20 days ([Hu et al.,](#page1) [2017a](#page1); [Tombuloglu et al., 2019](#page1)). Sampling was performed before flowering stage. Fresh sampled leaves were kept in liquid nitrogen for enzyme assays. The root and shoot length and fresh weight of root and stem were measured immediately. The dry weight of plants was mea-sured after drying samples in an oven held at 75 °C for 72 h ([Epstein and](#page1) [Rains, 1987](#page1)). The leaf area index was determined using Compueye software.

2.3. Determination of total phenolic content

For determining the phenolic content, the modified method of [Elzaawely and Tawata (2012a)](#page1) was used. Briefly, dry leaf tissue (0.1 g) was homogenized in 10 mL of 80 % methanol. The homogenate was centrifuged at 10,000 g for 15 min. To 0.2 mL of extract, 0.5 mL of distilled water and 0.5 mL Folin Ciocalteu reagent were added. After 5 min, 1 mL of 7% Na2CO3 was added to the mixture. The samples were incubated at room temperature for 90 min; the reaction mixture ab-sorbance was measured at 765 nm with a spectrophotometer (Dyna-mica HALO XB-10 UV–VIS single beam). Gallic acid (0–500 mg L−1) was used as a standard and the total phenolic compounds were ex-pressed as equivalents of gallic acid/ dry weight (DW).

2.4. Determination of total flavonoid content

In order to evaluate the flavonoid content, the modified method of [Elzaawely and Tawata (2012b)](#page1) was used. Briefly, dry leaf tissue (0.1 g) was homogenized in 1 mL of a solution containing flavonoids, 0.7 mL of 5% (w/w) NaNO2, and 10 mL of 30 % (v/v) ethanol and stirred for 5 min, then, 0.7 mL of 10 % AlCl3 (w/w) was added and the mixture was stirred up. After 6 min, 5 mL of 1 mol/l NaOH was added to

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homogenate. Subsequently, the solution was diluted to 25 mL with 30

* (v/v) ethanol prior to the measurement. After 10 min standing, the absorbance of the solution was measured at 500 nm with a Dynamica HALO XB-10 UV–vis single beam spectrophotometer. Catechin (0−0.004 mg L−1) was used as a flavonoid standard and the results were expressed as equivalents of catechin/ dry weight (DW).

2.5. Determination of anthocyanin content

The total monomeric anthocyanin content was determined using a spectrophotometric pH-diﬀerential method described by [Lee et al.](#page1) [(2005)](#page1) and [Humadi and Istudor (2009)](#page1). 1 mL of extract was leveled oﬀ to 10 mL using buﬀer solution with pH 1. A second 1 mL of extract was diluted to 10 mL with buﬀer solution with pH 4.5. The flasks were left at room temperature for 15 min, then, the absorbance was determined at 520 and 700 nm. The total anthocyanin content was expressed as cyanidin-3-glucoside equivalents as in the following equations.

Absorbance (A) = (A520 pH 1– A700 pH 1) – (A520 pH 4.5 – A700 pH 4.5)

Total anthocyanin (mg L−1) = (A/ 26,900) (103) (445.2) (5)

2.6. Determination of ascorbic acid content

Ascorbic acid (AsA) was isolated with 6% trichloroacetic acid from 100 mg of frozen leaf tissue. 4 mL of the extract was mixed with 2% dinitrophenylhydrazine (2 mL), followed by adding 1 drop of 10 % thiourea solution (in 70 % ethanol). The mixture was boiled for 15 min in bain-marie and cooled to room temperature. Afterwards, 5 mL of 80

* (v/v) H2SO4 was added to the mixture at 0 °C. The absorbance of the solution containing Hydrazone complex was determined at 530 nm using a spectrophotometer ([Mukherjee and Choudhuri, 1985](#page1)). Ascorbic acid solutions (0–600 mg mL−1) was used as a standard and the as-corbic acid was expressed as equivalents of ascorbate/ dry weight (FW).

2.7. Assay of enzymes activity

Fresh shoots and roots (0.5 g) were homogenized under ice-cold conditions in 10 mL of 50 mM phosphate buﬀer (pH 7.0), 1.0 % (w/v) polyvinilpyrrolidone (PVP), and 10 mM of Ascorbate (AsA). The homogenate was centrifuged at 15,000 g for 15 min. The supernatant was used for the enzyme assay.

APX activity was measured using spectrophotometer at 290 nm via decrease in AsA content, 1 min after addition of H2O2. The assay was carried out at 25 °C in a reaction mixture containing 50 mM of po-tassium phosphate (pH 7.0), 0.5 mM of AsA (extinction coeﬃcient 2.8 mmol/cm), 0.1 mM of H2O2 and 10 μl of enzyme extract ([Nakano](#page1) [and Asada, 1981](#page1)).

CAT activity was determined by monitoring H2O2 consumption (extinction coeﬃcient 39.4 mmol/cm) at 240 nm in 3 mL of reaction mixture containing 50 mM of phosphate buﬀer (pH 7.0), 15 mM of H2O2 and 80 μl of enzyme extract ([Aebi, 1984](#page1)).

GPX activity was analyzed according to [Monteiro et al. (2011)](#page1). The reaction medium contained 1 mL of phosphate-citrate buﬀer (0.2 M sodium phosphate dibasic; 0.1 M citric acid) pH 5.0, 0.5 % (v/v) guaiacol, 0.1 mM H2O2 and 2.5 μl enzyme extract. After completing homogenization, the samples were incubated at 30 °C for 15 min. The reaction was stopped by rapid cooling in an ice bath, followed by ad-dition of sodium metabisulphide 2% (w/v). After 10 min, GPX activity was determined by monitoring the absorbance at 450 nm.

GR activity was assayed with spectrophotometer, according to the method described by [Martins et al. (2011)](#page1), at 30 °C in a mixture con-taining 1 mL of 100 mM potassium phosphate buﬀer (pH 7.5) with 1 mM 5, 5- dithiobis- (2-nitrobenzoic acid), 1 mM GSSG and 0.1 mM NADPH. The reaction was initiated by adding 80 μL of plant extract.

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The reduction rate of GSSG was followed by measuring the increase in absorbance at 412 nm over 1 min.

2.8. Statistical analysis

All parameters assayed, were measured in three repetitions. The experimental design was Two-way and completely randomized, and data subjected to analysis of variance using SPSS for windows (version 21). Mean values and their standard errors were calculated from three replications. Diﬀerences between means were determined by Duncan’s multiple range tests (P ≤ 0.05). The diagrams were drawn by using Excel software.

3. Results

3.1. Plant growth analysis

The results of analysis of variance revealed that salinity stress aﬀects LA, SL, RL, SFW, RFW and RDW (P ≤0.01), as well as SDW (P ≤ 0.05). Eﬀect of NPs on SL and RFW and SFW were not significant, in contrary, iron oxide NPs spraying was showed a significant eﬀect on LAi, SDW, RDW (P ≤ 0.01) and RL (P ≤0.05). Interaction between salinity and NPs was not significant on all of the above mentioned traits except on LAi ([Table 1](#page1)).

It was observed that 0 mM NaCl + 90 ppm treatment caused an increase in SL, RL, SDW, RDW, SFW, RFW and LAi about 14.2, 26.5, 66, 67.2, 46, 40.6 and 17.7 %, respectively, compared to control plants. The lowest SL and RL, SFW and RFW, SDW and RDW were obtained in 100 mM NaCl + 90 ppm NPs treatment about 25.5, 10.2, 33.8, 58.5, 32.7 and 37.7 % decrease, respectively, compared to control plants ([Table 2](#page1)).

3.2. Total phenolic content

According to analysis of variance, salinity stress significantly af-fected the total phenolic content (P ≤ 0.01) by inducing secondary oxidative stress ([Table 3](#page1)). Applying iron oxide NPs had significant ef-fect on total phenolic content of root (P ≤ 0.01) and shoot (P ≤ 0.05). However, the interaction between Salinity × NPs was not significant on the total phenolic content of shoot ([Table 3](#page1)).

The lowest total phenolic content in shoot and root was observed in 0 mM NaCl + 30 NPs treatment while the highest total phenolic con-tent in shoot and root was obtained in 100 mM NaCl + 90 ppm NPs and 100 mM NaCl + 60 ppm NPs treatments, respectively, which did not have any statistically significant diﬀerence with other levels of NPs in 100 mM salinity stress ([Fig. 2](#page1)A, B).

3.3. Total flavonoid content

According to variance analysis results, the eﬀect of salinity stress on flavonoid content of shoot and root (P ≤ 0.01) was significant. Spraying iron oxide NPs had a significant eﬀect on flavonoid content of

Table 1

shoot (P ≤ 0.01) and root (P ≤ 0.05). Interaction between salinity × NPs on flavonoid content of root (P ≤ 0.05) was significant while its eﬀect on flavonoid content of shoot was not significant ([Table 3](#page1)).

Minimum and maximum contents of flavonoid in shoot were ob-tained in 0 mM NaCl + 60 ppm NPs and 100 mM NaCl + 0 ppm NPs treatments, respectively. The minimum content of flavonoid in root was recorded in control plant (without NaCl and NPs) and the maximum content of flavonoid was observed in 100 mM NaCl + 90 ppm NPs treatment ([Fig. 2](#page1)C, D).

3.4. Anthocyanin content

The results of analysis of variance exhibited that salinity stress significantly aﬀected the anthocyanin content of root and shoot (P ≤ 0.01). Spraying iron oxide NPs had a significant eﬀect on antho-cyanin content of root (P ≤0.05). Interaction of salinity and NPs on anthocyanin content was not significant ([Table1](#page1)).

Minimum and maximum contents of anthocyanin in shoot were found in 0 mM NaCl + 30 ppm NPs and 100 mM NaCl + 90 ppm NPs treatments, respectively. The minimum content of anthocyanin in root was observed in 0 mM NaCl + 90 ppm NPs treatment and the max-imum content was observed in control plants as well as in 100 mM NaCl + 60 ppm NPs treatment ([Fig. 2](#page1)E, F).

3.5. Ascorbic acid content

The obtained outcomes from analysis of variance revealed that salinity stress significantly aﬀected (P ≤ 0.01) AsA content in root and shoot tissues. Spraying iron oxide NPs had a significant eﬀect on AsA content of shoot (P ≤0.01). The interaction of salinity × NPs on AsA content of root (P ≤ 0.01) and shoot (P ≤ 0.05) was significant ([Table 3](#page1)).

The lowest ascorbate content in root and shoot was recorded in 0 mM NaCl + 60 ppm NPs treatment, and the highest amount was achieved in 100 mM NaCl + 0 ppm iron oxide NPs and also in 50 mM NaCl + 90 ppm NPs treatments ([Fig. 2](#page1)G, H).

3.6. Activity of antioxidant enzymes

Removing free radicals is another natural reaction of plants under oxidative stresses. In this study, it was evaluated by determining the level of activity of four antioxidant enzymes including CAT, GPX, APX and GR. Analysis of variance showed that salinity stress had a sig-nificant eﬀect (P ≤0.01) on all antioxidant enzymes activities both in shoot and root tissues. Moreover, spraying iron oxide NPs solution is significantly eﬀective on the activity level of antioxidant enzymes GPX,

|  |  |  |  |
| --- | --- | --- | --- |
| APX, GR and CAT in root | | | and shoot. The interaction of iron oxide |
| NPs × Salinity | | on activity | of enzymes APX, CAT, and GR in root |
| (P ≤ | 0.01) and | activity of | enzymes GPX, APX, and GR in shoot |
| (P ≤ | 0.05) was significant ([Table 3](#page1)). | | |

At simultaneous application of NaCl and iron oxide NPs, the

Analysis of variance plant growth parameters (Shoot length (SL), Root length (RL), Shoot Dry Weight (SDW), Root Dry Weight (RDW), Shoot Fresh Weight (SFW), Root Fresh Weight (RFW) and Leaf Area index (LAI)) in Dracocephalum moldavica L. treated with iron oxide NPs under salt stress conditions.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Source of variation | df |  |  |  | Mean Square |  |  |  |
|  |  | Shoot Length | Root Length | Shoot Dry Weight | Root Dry Weight | Shoot Fresh Weight | Root Fresh Weight | Leaf Area index |
|  |  | (SL) | (RL) | (SDW) | (RDW) | (SFW) | (RFW) | (LAI) |
|  |  | (cm) | (cm) | (g) | (g) | (g) | (g) | (cm2) |
|  |  |  |  |  |  |  |  |  |
| Salt | 2 | 126.69\*\* | 77.861\*\* | 0.029\* | 0.005\*\* | 4.308\*\* | 2.137\*\* | 171567.486\*\* |
| Nanoparticle | 3 | 11.954ns | 23.926\* | 0.044\*\* | 0.001\*\* | 0.553ns | 0.122ns | 204609.902\*\* |
| Salt × Nanoparticle | 6 | 22.065ns | 5.898ns | 0.014ns | 0.000ns | 0.877ns | 0.574ns | 16024.196\*\* |
| Error | 24 | 14.056 | 4.243 | 0.008 | 0.000 | 0.614 | 0.232 | 4127.325 |

ns: non-significant, \* and \*\*: significantly at 0.05 and 0.01 probability level, respectively. df: degree of freedom.

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Table 2

Plant growth parameters changes (Shoot length, Root length, Shoot Dry Weight, Root Dry Weight, Shoot Fresh Weight, Root Fresh Weight and Leaf Area index) of Dracocephalum moldavica L. plants treated with foliar application of Iron oxide NPs under salt stress conditions.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Salinity | Fe2O3 nanoparticle | Shoot Length | | Root Length | Shoot Dry Weight | Root Dry Weight | Shoot Fresh Weight | Root Fresh Weight | Leaf Area |
| (mM) | (ppm) | (cm) | | (cm) | (g) | (g) | (g) | (g) | (cm2) |
|  |  |  |  |  |  |  |  |  |  |
|  | 0 | 32.66ab | ± 2.4 | 16.33ab ± 1.45 | 0.421bc ± 0.090 | 0.061bcd ± 0.002 | 3.224bc ± 0.599 | 1.577b ± 0.163 | 1450.03c ± 27.94 |
| 0 | 30 | 31.66abc | ± 1.2 | 20.33ab ± 4.05 | 0.417bc ± 0.058 | 0.093abc ± 0.009 | 3.833ab ± 0.312 | 1.383b ± 0.514 | 1671.10ab ± 13.96 |
|  | 60 | 31.33abc | ± 3.38 | 21.33a ± 1.96 | 0.375bc ± 0.059 | 0.084ab ± 0.010 | 3.154bc ± 0.599 | 1.373b ± 0.335 | 1404.58cd ± 23.35 |
|  | 90 | 37.33a | ± 0.66 | 20.66ab ± 0.88 | 0.699a ± 0.044 | 0.102a ± 0.010 | 4.708a ± 0.279 | 2.218a ± 0.266 | 1719.63a ± 0.970 |
|  | 0 | 25.33bc | ± 1.2 | 18.33ab ± 2.33 | 0.426bc ± 0.037 | 0.044cd ± 0.005 | 3.004bc ± 0.516 | 0.893b ± 0.175 | 1314.16def ± 32.97 |
|  | 30 | 30.33bc | ± 2.02 | 19.33ab ± 2.72 | 0.415bc ± 0.029 | 0.063bcd ± 0.009 | 2.688bc ± 0.212 | 1.033b ± 0.196 | 1570.50b ± 51.02 |
| 50 | 60 | 28.33bc | ± 2.96 | 16.66ab ± 2.02 | 0.358bc ± 0.047 | 0.059bcd ± 0.006 | 2.974bc ± 0.381 | 0.987b ± 0.227 | 1274.63ef ± 64.76 |
|  | 90 | 30.00bc | ± 2.64 | 19.00ab ± 0.57 | 0.469b ± 0.056 | 0.066bcd ± 0.003 | 3.190bc ± 0.439 | 0.996b ± 0.212 | 1680.80ab ± 50.49 |
|  | 0 | 25.66bc | ± 1.20 | 17.00ab ± 1.00 | 0.283c ± 0.038 | 0.038d ± 0.003 | 2.630bc ± 0.167 | 0.653b ± 0.174 | 1293.00def ± 10.05 |
|  | 30 | 28.33bc | ± 0.88 | 15.00ab ± 1.04 | 0.417bc ± 0.071 | 0.051bcd ± 0.005 | 3.156bc ± 0.747 | 0.932b ± 0.436 | 1347.03cde ± 14.72 |
| 100 | 60 | 29.33bc | ± 3.48 | 18.00ab ± 1.15 | 0.371bc ± 0.034 | 0.050cd ± 0.005 | 2.278c ± 0.363 | 1.088b ± 0.108 | 1212.90f ± 7.125 |
|  | 90 | 24.33c | ± 1.20 | 14.66b ± 0.88 | 0.421bc ± 0.010 | 0.044cd ± 0.002 | 2.132c ± 0.438 | 0.735b ± 0.232 | 1355.96cde ± 48.51 |

Values represent the means of three replications ± standard error (n = 3, Two-way ANOVA). Diﬀerent letters within a column indicate significant diﬀerences (P ≤ 0.05) according to Dancan’s test.

minimum and maximum levels of GPX activity of root and shoot were observed in 0 mM NaCl + 60 ppm NPs and 100 mM NaCl + 0 ppm NPs treatments, respectively ([Fig. 3](#page1)E, F). The minimum activity of enzymes: APX ([Fig. 3](#page1)A, B), CAT ([Fig. 3](#page1)C, D), and GR ([Fig. 2](#page1)G, H) in root and shoot was found in 0 mM NaCl + 30 ppm iron oxide NPs treatment. The maximum activity level of APX in shoot, CAT in root and shoot, and GR in root was observed in 100 mM NaCl + 60 ppm NPs treatment; how-ever, the maximum activity level of APX in root was seen in 100 mM NaCl + 0 ppm NPs treatment. The maximum activity level of GR in shoot was observed in 100 mM NaCl + 90 ppm iron oxide NPs treat-ment ([Fig. 3](#page1)G).

4. Discussion

Herein, by increasing the salt concentration, RL, SL, RFW and SFW showed a decreasing trend, so that the highest and lowest means were obtained in control plants and the plants treated with 100 mM NaCl, respectively. The accumulation of salt in the solution of soil decreases

Table 3

the osmotic potential of soil solution, therefore, the plant encounters a serious problem in water absorption resulting a physiological drought. On the contrary, due to the presence of toxic ions in the soil solution, the plant is exposed to the toxicity of these ions. Such eﬀects can reduce cell inflammation, photosynthesis, enzyme activity and eliminate ionic balance due to the inadequate transfer of ions ([Munns, 2005](#page1)). [Kwon](#page1) [et al. (2019)](#page1) suggested that a decrease in growth caused by salinity was due to the reduction rate of photosynthesis per unit area in carnation. In this regard, [Munns (2002)](#page1) observed that salinity decreased Alfalfa leaf area, resulting in decreased amount of photosynthesis. However, the reduction in root growth may not only aﬀect the transfer of water and nutrition, but also aﬀects the growth of shoot by causing a disorder in hormonal balance. As is well known, the root is the source of photo-synthetic material depletion, any decline in growth means the inability of root in consumption of photosynthetic materials, which leads to creation of inhibitory feedback system and decrease in photosynthesis, as a result, the growth of the shoot will be lessen ([Bahar et al., 2005](#page1)).

Generally, the eﬀects of salinity stress on plant growth and its

Analysis of variance for enzymatic and non-enzymatic antioxidant in Dracocephalum moldavica L. treated with iron oxide NPs under salt stress conditions.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  |  |  | Mean Square | |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| Source of variation | df | Total phenolic | Total phenolic | Flavonoid | Flavonoid | Anthocyanin | Anthocyanin | Ascorbate | Ascorbate | |
|  |  | content of | content of | content of | content of | content | content | content of shoot | content of | |
|  |  | shoot | root | shoot | root | of shoot | of root | (μg g -1 FW) | root | |
|  |  | (mg g -1 DW) | (mg g -1 DW) | (mg g -1 DW) | (mg g -1 DW) | (mg g -1 DW) | (mg g -1 DW) |  | (μg g -1 FW) | |
|  |  |  |  |  |  |  |  |  |  |  |
| Salt | 2 | 1985.915\*\* | 574.999\*\* | 0.158\*\* | 0.013\*\* | 0.396\*\* | 0.399\*\* | 0.001\*\* | 0.000\*\* |  |
| Nanoparticle | 3 | 761.455\* | 80.675\*\* | 0.062\*\* | 0.018\* | 0.082 ns | 0.070\* | 0.000\*\* | 2.277E-5 \*\* | |
| Salt×nanoparticle | 6 | 69.742ns | 19.092 ns | 0.006ns | 0.001\* | 0.008 ns | 0.009 ns | 5.59E-5\* | 4.530E-5\*\* | |
| Error | 24 | 34.985 | 12.994 | 0.006 | 0.000 | 0.030 | 0.036 | 1.713-5 | 1.085E-5xxx | |
|  |  |  |  |  |  | |  |  |  |  |
|  |  |  |  |  | Mean Square | |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| Source of variation | df | GPX activity of | GPX activity of | APX activity of | APX activity of | CAT activity of | CAT activity of | GR activity of | GR activity of | |
|  |  | shoot | root | shoot | root | shoot | root | shoot | root | |
|  |  | (μmol min-1/ g | (μmol min-1/ g | (μmol min-1/ | (μmol min-1/ | (μmol min-1/gFW) | (μmol min-1/ | (μmol min-1/ | (μmol min-1/ | |
|  |  | FW) | FW) | gFW) | gFW) |  | gFW) | gFW) | gFW) | |
|  |  |  |  |  |  |  |  |  |  |  |
| Salt | 2 | 55.731\*\* | 21.883\*\* | 0.046\*\* | 0.018\*\* | 1209.290\*\* | 432.701\*\* | 0.025\*\* | 0.008\*\* |  |
| Nanoparticle | 3 | 37.684\*\* | 18.665\*\* | 0.035\*\* | 0.009\*\* | 86.361\* | 99.033\*\* | 0.015\*\* | 0.003\*\* |  |
| Salt×nanoparticle | 6 | 7.108\* | 1.313 ns | 0.006\* | 0.006\*\* | 36.870ns | 39.849\*\* | 0.000\* | 0.001\*\* |  |
| Error | 24 | 2.172 | 1.301 | 0.001 | 0.000 | 21.266 | 5.082 | 7.631E-5 | 3.331E-5 | |
|  |  |  |  |  |  |  |  |  |  |  |

ns: non-significant, \* and \*\*: significant at 0.05 and 0.01 probability level, respectively. df: degree of freedom, GPX: guaiacol peroxidase, APX: ascorbate peroxidase,

CAT: catalase, GR: glutathione reductase.

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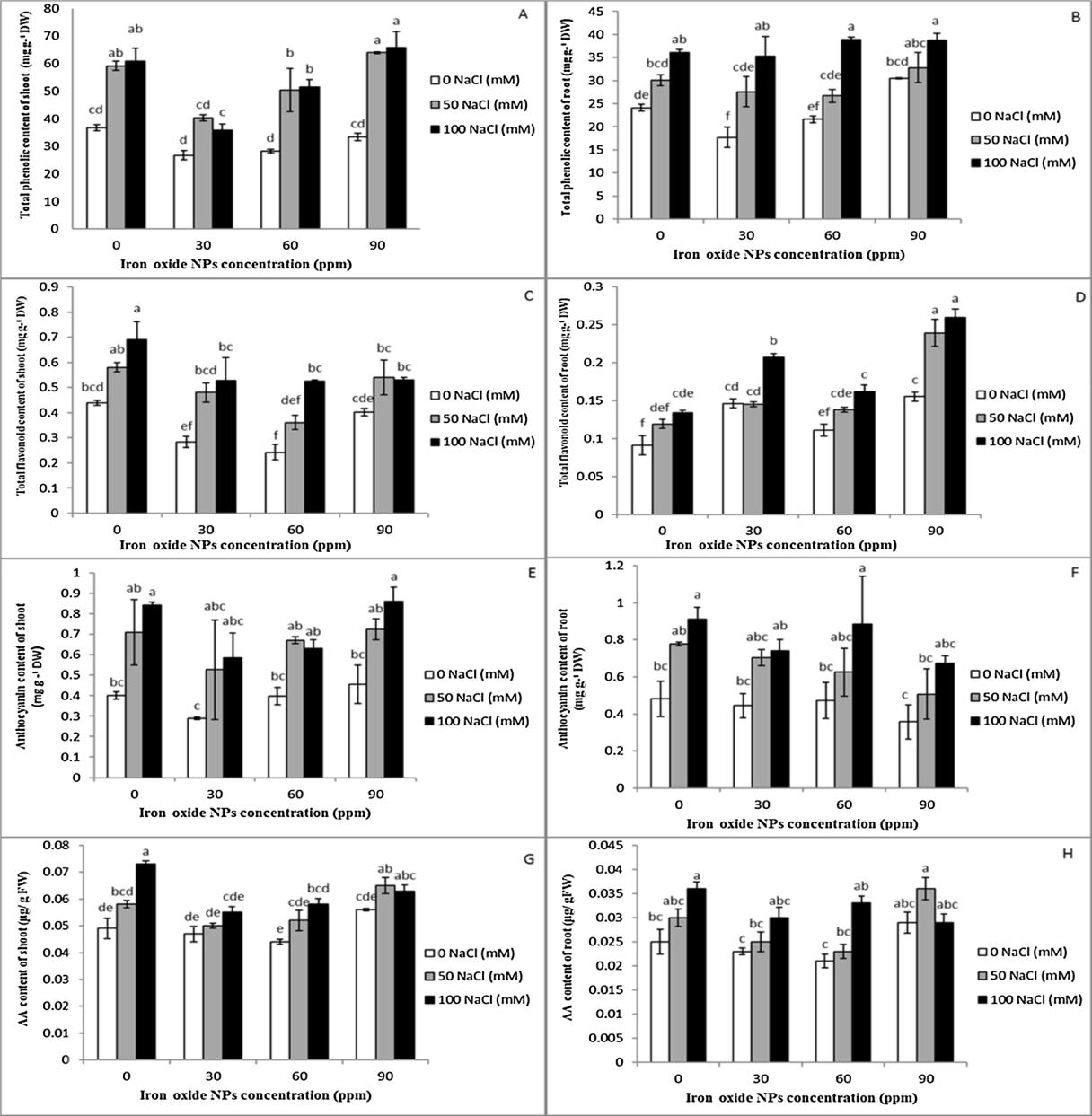


Fig. 2. Non-enzymatic antioxidant changes in shoot (total phenolic (A), total flavonoid (C), anthocyanin (E) and ascorbic acid (G) content) and root (total phenolic (B), total flavonoid (D), anthocyanin (F) and ascorbic acid (H) content) of Dracocephalum moldavica L. plants treated with foliar application of Iron oxide NPs under salt stress conditions. Bars within a chart the same lower-case letter are not significantly diﬀerent at P ≤ 0.05. Bars are the means of three replications ± standard error (n = 3). Diﬀerent letters above bars show a significant diﬀerence (Two-way ANOVA Duncan’s test; P ≤ 0.05).

functions are complex. The decrease in plant growth can be due to a change in the allocation of materials such as photoassimilates to the roots, which leads to a decrease in the growth of shoot, especially leaves, partial or total closure of the stomata. This might also because of the direct eﬀect of salt on the photosynthetic system, or its eﬀect on the ionic balance ([Brugnoli and Bjorkman, 1992](#page1); [Bose et al., 2017](#page1)). De-crease in leaf area is probably owing to the reduction of relative water content in leaf leading to a decrease in the size and the division of meristem cells, eventually, causing a decline in leaf growth, inhibiting leaf production, accelerating aging and thus loss of leaves ([Lobato et al.,](#page1) [2008](#page1); [Osuagwu et al., 2010](#page1)). The results of experiments ([Goksoy et al.,](#page1) [2004](#page1); [Karam et al., 2007](#page1)) showed that LAi decreases under stress conditions. Also, high salt accumulation in the soil causes the root length decline and ultimately reduces the root production.

In this study, foliar application of NPs increased RL, SL, SFW, RFW,

SDW and RDW, but this increase was not significant. Root dry weight in all three levels of iron oxide NPs exhibited an important increase in comparison with control plants.

[Jabeen and Ahmad (2011)](#page1) have reported that foliar application of potassium, iron and boron elements increase plant height, dry weight of shoot in sunflower under salinity stress. Also in another experiment ([Zayed et al., 2011](#page1)), it was observed that, spraying iron, zinc and manganese increased the height, dry weight of shoot and chlorophyll content of rice under salinity stress. Due to the role of iron in the chlorophyll structure and plant photosynthesis system, application of iron nano-chelate at appropriate levels improves photosynthesis and produce the material in the plant by increasing iron, and finally in-creases the dry weight of the plant. Studies have shown that iron spray application on Mentha pepermint L. has significantly increased the dry weight of plant in comparison with control plants ([Asad and Rafique,](#page1)

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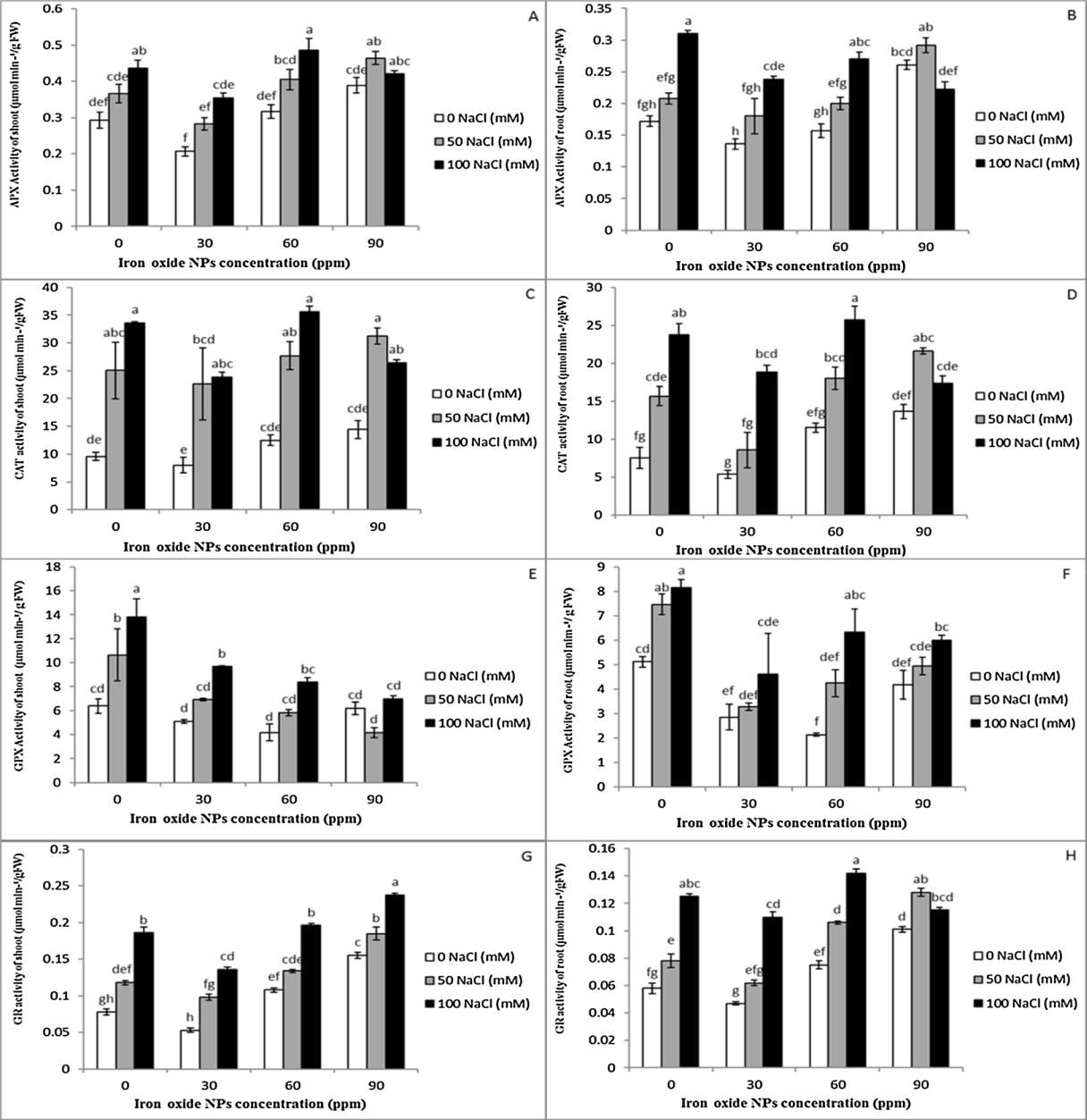


Fig. 3. Enzymatic antioxidant changes in shoot (APX (A), CAT (C), GPX (E) and GR (G) activity) and root (APX (B), CAT (D), GPX (F) and GR (H) activity) of Dracocephalum moldavica L. plants treated with foliar application of Iron oxide NPs under salt stress conditions. Bars within a chart the same lower-case letter are not significantly diﬀerent at P ≤0.05. Bars are the means of three replications ± standard error (n = 3). Diﬀerent letters above bars show a significant diﬀerence (Two-way ANOVA Duncan’s test; P ≤0.05).

[2000](#page1)). In another study, the highest biomass of wheat with 4 Kg ha−1 iron foliar application was observed. In as much as the structural role of micronutrients such as iron in some enzymes as well as their eﬀective role in the synthesis of proteins, applying these elements enhance the yield and also increase the resistance of plants to environmental stresses ([Ruiz et al., 2000](#page1)). [Plaksenkova et al. (2019)](#page1) reported that using iron oxide NPs increased RL and SL, yield and quality of rocket seedlings and also improved the ability of plants to encounter with environmental stresses.

Phenolic compounds as antioxidants have inhibitory properties to protect plants against oxidative damage ([Singh et al., 2018](#page1)). Anti-oxidant activities of phenolic compounds are mainly caused by their oxidization-reduction characteristic which has an important role in absorbing and neutralizing free radicals ([Joyce et al., 2005](#page1)). [Klimczak](#page1) [et al. (2007)](#page1) stated that treating plants with salinity causes

accumulation of phenolic compounds, hence, increase the antioxidant activity which is in agreement with results presented in this study. [Valifard et al. (2014)](#page1) reported that by increasing salinity levels, the phenolic content of Salvia increased. However, the phenolic content reduced in the highest level of salt stress. They stated that, in spite of synthesis induction of phenolic compounds in salinity treatments, any reduction in phenolic content may be probably result of the severe salinity stresses negative eﬀect on antioxidant features of the plants that decrease the phenolic compounds and antioxidant capacity conse-quently ([Sidsel Fiskaa et al., 2009](#page1)).

[Apel and Hirt (2004)](#page1) stated that any increase in phenolic content in plants under stress is resulted from the induction of particular defense mechanisms in plants against oxidative damages from ions, therefore, cytoplasmic structures and chloroplast are protected. The specification of phenols is because of their hydroxyl and carboxyl groups which are

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able to link to copper and iron particularly ([Sakihama and Yamasaki,](#page1) [2002](#page1)). Phenols may deactivate iron ions by chelating them and de-crease superoxide level derived from Fenton reaction as the main source of ROSs ([Winkel-Shirley, 2002](#page1)).

Flavonoids also have antioxidant feature and play important role in adjusting enzymatic activities and producing primary metabolites. These compounds are accumulated in plant tissues and by means of existing hydroxyl groups in their structures; they can act as neutralizers of free radicals ([Grace and Logan, 2000](#page1)). The amount of phenolic compounds in barley seedlings is increased under salinity stress. Re-duction of germination and growth associated with salinity-induced disorders in metabolic processes cause an increase in phenolic com-pounds, flavonoid ([Ayaz et al., 2000](#page1)) and oxidative enzymes ([AbdElgawad et al., 2016](#page1)) that all confirm our results.

Flavonoids prevent oxidative stress directly by inducing the reduc-tion reactions or indirectly by chelation of iron because of their anti-oxidative role. It seems that any increase in non-enzymatic antioxidant pigments following by iron solution spraying; prevent oxidative stresses by scavenging the ROSs. Therefore, the increase of flavonoids level can be considered as a salt tolerance inducing factor in Moldavian balm and spraying iron NPs solution increase the flavonoid content. Researches have reported that in Melissa oﬃcinalis L. spraying iron solution has a positive eﬀect on increasing rosmarinic acid (a type of flavonoid) ([Posmyk et al., 2009](#page1)). In another study in saﬄower, it was observed that the application of iron chelates in high amount, increase the fla-vonoid content ([Ghorbanpour, 2015](#page1)). It seems that determining the desirable level of iron has an important role in increasing or decreasing flavonoid level in plants. Application of NPs can lead to an increase in accumulation of phenolic content ([Chung et al., 2019](#page1)). [Nourozi et al.](#page1) [(2019)](#page1) stated that the reason for the increased amount of flavonoid and phenolic in Dracocephalum kotschyi after iron NPs treatment is an in-crease in expression of genes, involved in phenylpropanoid pathway.

Anthocyanin facilitates entrance of toxic sodium and chlorine ions into vacuoles and collecting them from other parts as a consequence. These compounds, as the protective system of plant, prevent the per-oxidation of lipids by increasing the oxidative stress ([Mittler, 2002](#page1)). On the other hand, in high concentrations, salinity stress aﬀects enzymes involving in photosynthesis and also photosynthetic membranes; which results a disordering in chloroplast function and structure which may lead to more sensitivity to higher levels of light. Therefore, producing pigments such as anthocyanin which protects plants against light can protect them from oxidative damages induced by light either. This can be a strong reason for gradual increasing in anthocyanin content of leaves simultaneously with increasing in salt concentration ([Mittler,](#page1) [2002](#page1)). Results showed that under salinity stress, the amount of an-thocyanin concentration is increased in diﬀerent concentrations of so-dium chloride compared to control samples. These results correspond to a report by [Kong et al. (2003)](#page1). They presented the amount of antho-cyanin in plants depending on growth conditions and environment, and it was shown that its amount increases as a result of environmental stresses in plants.

Many reports have shown the high anthocyanin content in salt tolerant plants ([Wahid and Ghazanfar, 2006](#page1)). [Garriga et al. (2014)](#page1) investigated the salinity stress in wild strawberry, investigations af-firmed that an increase in the amount of anthocyanin occurs under salinity conditions, which is in agreement with the results reported in the present study.

In an experiment, the eﬀect of salinity on anthocyanin in root, hy-pocotyl and cotyledon of tomato and red cabbage were studied and it was observed that by increasing salinity, anthocyanin production in-duced and its amount was increased. Generally, any change in plants pigments is resulted from their defensive mechanisms against salinity. This hypothesis can be stated that enhancement in anthocyanin bio-synthesis in root, stem, and particularly in plants leaf improves re-sistance against environmental stresses. Also, there is a significant correlation between increase in the amount of anthocyanin and

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antioxidant enzymes ([Eryilmaz, 2006](#page1)).

Studies have shown that spraying iron sulfate solution in Berberis vulgaris L. increased the anthocyanin content ([Kalinova and Vrchotova,](#page1) [2011](#page1)). Increasing the non-enzymatic pigments using iron, prevents chlorophylls destruction. In another study on saﬄower, foliar spraying and soil consumption of iron chelate solution enhanced the amount of anthocyanin content in leaf compared to the control plants ([Ghorbanpour, 2015](#page1)). It seems that using iron nano-chelate has a po-sitive eﬀect on the amount of anthocyanin which may increase plant photosynthesis by preventing damages to chlorophyll. However, the increase in iron level may sometimes decrease the amount of antho-cyanin which might be because of high iron accumulation in the plant. Researchers have reported that phenolic and anthocyanin compounds are important factors confronting free radicals in stresses related to metals ([Manquián-Cerda et al., 2016](#page1); [Chen et al., 2019](#page1)). Therefore, the increase in anthocyanin is protective state for scavenging free radicals and preventing destruction of biological molecules. According to [Mahmoud et al. (2019)](#page1) foliar application of ZnO and iron oxide NPs increased the secondary metabolites such as anthocyanin of Raphanus sativus plants. Carbohydrates amounts enhancement aﬀected by NPs may one of the reasons to increasing of anthocyanin content ([Mahmoud](#page1) [et al., 2019](#page1)).

AsA is another antioxidant substance which plays an important role in ascorbate-glutathione cycle; a very important cycle in decomposing H2O2 ([Kaya and Yigit, 2014](#page1)). Our findings indicate a decrease in AsA content under the influence of iron oxide NPs ([Fig. 2](#page1)G, H). The content of AsA in shoot is reduced by 10.2 % in 0 mM NaCl +60 ppm NPs treatment, while salinity stress enhanced AsA content of shoot and root. According to [Olkhovych et al. (2016)](#page1), copper NPs led to reduce in the amount of AsA in Pistia stratiotes L. plants. It has been revealed that the activity of enzymes related to AsA metabolism is inhibited by metal ions ([Olkhovych et al., 2016](#page1)). A decrease in AsA content in bryophyte plants exposed to ZnO NPs ([Motyka et al., 2019](#page1)) was reported.

In the present study, by applying salinity stress, the activity of APX, CAT, GPX, and GR enzymes was increased. The improve in CAT enzyme activity in response to salinity stress has also been reported by other researches ([Baily, 2004](#page1)). This enzyme helps plant to survive by re-moving diﬀerent types of reactive oxygen and preventing the cell wall destruction ([Jiang and Zhang, 2001](#page1)).

Since some researchers believe that the proteins synthesis is de-creased in high salinity stresses, then CAT enzyme activity might also be decreased at high stresses of salinity ([Khanna-Chopra and Selote,](#page1) [2007](#page1)). The results concerning to activity of GPX under salinity stress revealed that this enzyme acts as a defensive tool for inducing re-sistance against oxidative damage as a result of salinity stress in Mol-davian balm.

APX activity increased under salinity stress in tolerant varieties ([Moradi and Abdelbaghi, 2007](#page1)), but its activity did not change in sensitive varieties ([Demiral and Turkan, 2005](#page1)). In an experiment, [Alvesda Costa et al. (2005)](#page1) reported that under salinity stress the level of APX in diﬀerent varieties of sorghum is increased whereas the CAT activity is reduced. Even though the increase in peroxidase enzyme activity by salinity stress was reported in sugar beet ([Bor and Türkan,](#page1) [2003](#page1)) and rice ([Demiral and Turkan, 2005](#page1)), there is an evidence of the decrease in peroxidase enzyme activity under salinity stress ([Demiral](#page1) [and Turkan, 2005](#page1)). It seems that decrease in soluble protein content in leaf as a result of salinity, causes thylakoid and Calvin cycle proteins decrease, especially rubisco and some antioxidant enzymes ([Bor and](#page1) [Türkan, 2003](#page1)). Another investigation on the eﬀect of iron on medicinal plants (Bacopa monnieri L.) revealed that after 48 h treatment, perox-idase activity in leaf decreased while its activity increased in root, furthermore, it was found that APX content increased both in root and leaf compared to control plants ([Sinha and Saxena, 2006](#page1)).

GR enzyme is also one of the glutathione-ascorbate pathway en-zymes which reduces glutathione by consuming NADPH as electron donor ([Baily, 2004](#page1)). Glutathione-ascorbate cycle plays an important

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role in developing defense system against oxidative stress, in this re-spect, increase in the enzymes activity under stress conditions leads to a decrease in oxidative stress eﬀects ([Khanna-Chopra and Selote, 2007](#page1)). GR plays a key role in reducing hydrogen peroxide through Haliwell-Asada pathway ([Baily, 2004](#page1)). It has been reported that an increase in GR enzyme activity is related to salinity tolerance ([Yasar et al., 2013](#page1)).

Lack of iron in plants not only develops chlorosis, but also decreases certain enzyme activity such as CAT and peroxidase since these en-zymes have Heme group as a prosthetic group located in porphyrin playing crucial role in plant metabolism ([Anwar et al., 2018](#page1)). There-fore, it seems that using 50 kg iron chelate in one acre may decreases the negative and destructive eﬀects of drought stress. Using 100 kg iron chelate in 1 acre without drought stress condition had a positive eﬀect on the APX enzyme activity. [Ranieri et al. (2001)](#page1) reported that the peroxidase iso-enzymes activities in soybean and sunflower leaves grown in nutritional solution lacking iron, caused an important de-crease in scavenging of H2O2. Accordingly, it can be stated that the most important enzymes in the plants neutralizing hydrogen peroxide, i.e. CAT, peroxidase, and APX, are the ones containing iron element.

One of the reason of increasing Moldavian balm functions by con-suming iron NPs under salinity stress conditions can be the eﬀect of this element on the antioxidant enzymes activity. The results of diﬀerent experiments have shown that using micronutrient elements decreases the negative eﬀect of environmental stresses such as drought and sali-nity stress ([Wang et al., 2004](#page1)) since, micronutrient elements such as iron, zinc, copper, magnesium, and manganese, play as a cofactor in structure of some antioxidant enzymes. Therefore, when plants en-counter deficiency of these elements, the activity of antioxidant en-zymes is decreased and the sensitivity to environmental stresses is in-creased ([Agarwal et al., 2006](#page1)). Previous studies reported an enhancement of secondary metabolite production and antioxidant en-zyme activity by iron oxide NPs in Hyoscyamus reticulatus ([Moharrami](#page1) [et al., 2017](#page1)) and Citrus maxima plants ([Hu et al., 2017b](#page1)).

Generally, considering the findings of this experiment, increase in antioxidant enzymes activity as a result of consuming iron NPs, parti-cularly under salinity stress conditions, can maintain cell membrane. Nanoparticles can disrupt the chloroplast and mitochondrial electron transport system at high concentrations, which results production of ROSs ([Cvjetko et al., 2017](#page1)). Although ROSs produced by nanoparticles can damage proteins and DNA ([Belava et al., 2017](#page1); [Saha and Dutta](#page1) [Gupta, 2017](#page1)), they can play an important role in response to environ-mental stress ([Sharma et al., 2012](#page1)). In order to scavenging of ROSs, enzymatic and non-enzymatic antioxidant systems are activated and plants increase the rate of production of antioxidant molecules to cope up with environmental stresses ([Rastogi et al., 2017](#page1)). The increase of flavonoids, anthocyanin, phenolic compounds, carotenoids, and anti-oxidant enzymes are evidence for the relation between producing free radicals and increase in accumulation of these substances. Several re-ports indicated that the production of antioxidant compounds in the plant is increased under the influence of NPs ([Jiang et al., 2014](#page1); [Costa](#page1) [and Sharma, 2016](#page1)), indicating the regulation of the antioxidant system by the NPs. At appropriate NPs concentrations, there is a balance be-tween the production of ROSs and antioxidant activities, but with an increase in the concentration of NPs, this balance is disturbed and the ROSs destroy the macromolecules leading to cell death ([Sharma et al.,](#page1) [2012](#page1)).

The excessive amount of Fe2O3 NPs may stimulate ROSs production in plants, which is also known as a signaling molecule and stimulating antioxidant defense ([Rui et al., 2016](#page1)). However, by increasing iron NPs concentration (90 ppm), the level of enzymes activity, particularly in root, is decreased which shows ineﬃciency of antioxidant defense system at high concentration of NPs + NaCl. Considering the results mentioned above, it seems that in Moldavian balm plants, non-enzy-matic system is important in order to neutralize destructive eﬀect of stress. Therefore, when the non-enzymatic defense system is weakened, the enzyme defense system is activated and it helps to remove the ROSs

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and their destructive eﬀect on the plant. Actually, the capacity of plants in increasing antioxidant enzymes activity confronting stress eﬀects seems to be limited. In some studies, it has been shown that increased concentration of iron eventually leads to decrease in all antioxidant enzymes ([Schutzendubel and Polle, 2002](#page1)).

5. Conclusion

The results of the present study showed that application of iron oxide NPs simultaneously with salinity stress did not have a negative impact on plant growth and yield, whereas, improved the nutritional conditions of plant, reducing the adverse eﬀects of salinity stress. In this study, due to the positive eﬀect of iron oxide NPs on resistance against salt stress, the content of phenolic compounds and flavonoids increased, especially in the roots. Therefore, the medium salinity and Fe2O3 NPs can be introduced as eﬃcient inducers aﬀecting the biosynthesis of secondary metabolites in the Moldavian balm. Herein, by increasing the concentration of Fe2O3 NPs, antioxidant enzymes activity is decreased in plants under salinity stress. Considering the results mentioned above, it seems that in Moldavian balm, non-enzymatic system is important in order to neutralize destructive eﬀect of stress. Hence, when the non-enzymatic defense system is weakened, the enzyme defense system is activated, that helps to remove the free radicals and their destructive eﬀects on the plant. It can be concluded that appropriate and optimum levels of iron Fe2O3 NPs may be used as a source of Fe for Dracocephalum moldavica L. plants under saline conditions.

Credit author statement

Hanieh Moradbeygi: performed experimental activities, writing and original draft preparation. Rashid Jamei and Reza Heidari: designed and supervised the work and Reza Darvishzadeh: Data curation, re-viewing, editing and preparation of the final version of manuscript. All authors do discussion on the results. The authors accept full responsi-bility for the context of the manuscript.

Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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