Journal of Plant Process and Function, Vol. 9, No. 37, Year 2020

Research Article

Green synthesis of Au-Ag nanoparticles using *Mentha piperita* and effects of Au-Ag alloy nanoparticles on the growth of *Mentha piperita* under salinity stress

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(Received: 13/10/2019-Accepted:28/07/2020)

Abstract

Salinity stress reduces plant metabolic activity, which in turn reduces plant growth in saline environments. This study was aimed to investigate the effect of salinity stress as one of the limiting factors of plant growth and the effect of nanoparticles on stress conditions as one of the auxiliary factors in plant control against stressful conditions by studying physiological factors such as soluble sugars, chlorophyll, carotenoids, proline, anthocyanins, and antioxidant enzymes. For this purpose, a factorial experiment was conducted in a completely randomized design with three replications. The first factor is tension salinity with different concentrations of salt, including zero, 50, 100, and 150 mg per liter and the second factor includes the effect of Au-Ag nanoparticles on physiological parameters of *Mentha piperita* under salt stress. Stress was applied at the vegetative growth stage. The high amount of antioxidant compounds in mentha's extract provides a possibility of nanoparticle synthesis by mentha extract. In this study, Au-Ag alloy nanoparticles (NPs) which have consolidated properties of nanoparticles of Au and Ag synthesized by mentha extracts (green chemistry method) .The results of experiments on the *Mentha piperita* showed that salinity stress causes decrease in the length of root and shoot, fresh and dry weight of leaf and root, chlorophyll a and b, carotenoids, and anthocyanin content. Results also show that it causes increase activity of catalase and guaiacol peroxidase enzymes, soluble sugars amount in leaves and roots, and these effects show the greatest difference at a concentration of 150 mM NaCl.

Keywords: Nanoparticles, Salt, Au-Ag, Plant growth, Decrease

Introduction

Nowadays salinity has become one of the most serious environmental issues that has caused a great reduction in the growth and development of plant species. It appears that salinity is one of the major limiting factors for crop plants mainly in arid and semiarid regions of the world. The detrimental effects of high salinity on plants can be observed either at the whole plant level or at the death of plants and/or decrease in productivity (Parul Parihar *et al*., 2015). Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism are affected. The earliest response was a reduction in the rate of leaf-surface expansion followed by a cessation thereof as the stress intensifies (kare hartvig Jensen *et al*., 2012). Growth resumes when stress is reduced. Carbohydrates, which are essential for cell growth, are mainly obtained through the process of photosynthesis and their levels in plants that are exposed to salinity are greatly reduced. From the results of the studies, which looked at the effects of salt stress on growth, one can notice a connection between the decrease in plant length and the increase in the concentration of sodium chloride (Dalton *et al*., 1994). Many studies show the effect of salinity inhibition on biochemical processes. For example, photosynthesis can be evaluated through the effects of photosynthetic pigments. The results of specific studies show that salinity reduces the content of photosynthetic pigments in a treated plant (Mane *et al*., 2010).

Recently engineered nanoparticles (NPS) have been widely used in many industries worldwide. However, the significant impacts of metal nanoparticles on plant growth are of concern for food quality. Various studies show the toxic effects of NPS on seed germination, plant growth, nutrient uptake, and photosynthesis in different media such as agar nutrient solutions and soil (Siddiqui *et al.*, 2014). Nanotechnology can present a solution to increase the value of agricultural products, environmental problems, and nanomaterials. Because of their tiny size, they show unique characteristics (Bharathi *et al*., 2014). The addition of nanoparticles in a liquid changes their chemical physiological and transports characteristics compared to their base fluids such as enhancements of thermal conductivity. There is a variety of methods to synthesize Ag/Au nanoparticles (NPS) including physical and chemical methods (Karami *et al*., 2016). Although chemical routes are

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effective, these may suffer from toxicity due to chemicals used and the difficulty in removing them. Additionally, the chemical reagents used in these methods are hazardous to the environment. To avoid toxicity, the chemical-green synthesis method was developed. (Ruffini and Cremonini, 2009) .This method was for the biosynthesis of metal nanoparticles. It has been proposed as a cost-effective and environmentally friendly way of fabricating these materials. Synthesis Au-Ag NPS employing either microorganisms or plant extracts has emerged as an alternative approach. These biosynthetic methods have several benefits. They are simple, cost-effective methods that can give high yields and are environmentally friendly (Seif *et al*., 2011).

Materials and methods

Fresh leaves of mentha were collected from mountainous areas of Babol (120AMSL: above mean sea level) in October (vegetative state). Identification was performed in the biological laboratory of Mazandaran University. Their surface was cleaned with running tap water to remove mud and other contaminated organic contents. Then, they were washed again by double-distilled water and air-dried at room temperature. About 20 grams of finely cut leaves were kept in a beaker containing 250 ml of methanol. Then, the solvent was vapored and extracted by the Suxhle method for 30 minutes. The extract was cooled down and filtered with whatman filter paper no.1 and the extracts were stored in sealed glass vials in a refrigerator at 4-5°C to prevent changes in chemical composition.

For green synthesis of Au-Ag nanoparticles, 5 ml of $AgNo_3$ 1 mM and 5 ml of $AuCl_3$ 1 mM (purchased from Merck, Germany), were prepared in an erlen mayer flask. Then, 4.5 ml of plant extract was added to this solution and stirred for 30 minutes at 50°C. The reduction of Ag^+ and Au^{2+} to Ag0 and Au0 was confirmed by the color change of the solution from colorless to brown-red. Its formation was also confirmed by using UV-visible spectroscopy. To prove the synthesis of nano alloy Au-Ag, UV-Vis spectroscopy, TEM, and DLS images were taken. For the preparation of rhizomes, the pots were autoclaved before. The rhizomes of *Mentha piperita* were provided by Zarin Giyah Oromoiyeh company. General roles for investigating salinity stress and salinity treated by nano alloy Au-Ag effects, were as follow: Salinity stress was done in 3 different concentrations (50, 100, and 150 mM) and treated nano alloy Au-Ag (100 ppm). On the other hand, the effects of $AgNO_3$ and $AuCl_3$ (1 mM) as blanks were investigated. Planting environments of *Mentha piperita 's* rhizomes were divided into 5 categories: Control, salinity stress, salinity combined with Au-Ag nano-alloy, Au nitrate, and Ag chloride. To investigate the effect of salinity treatment with Au-Ag nanoparticles, Au nitrate, and Ag chloride on peppermint, rhizomes before planting were immersed for 90 minutes in Au-Ag nanoparticle solutions (100 ppm), and Au nitrate and Ag chloride (1 mM) and then prepared for implantation. Subsequent rhizomes and other rhizomes related to control salinity stresses were planted in pots with a diameter of 20 and a height of 18 cm, which were autoclaved for 4 hours at a temperature of 121°C and a pressure of 1 atmosphere. The soil inside each pot was a mixture of cultivated soil, sand, and rotten manure in a ratio of 6:3:1. After planting, the pots were exposed to light conditions for 16 hours of light and 8 hours of darkness and a temperature of 25°C in the growth room and they were irrigated every other day. 25 days after rhizomes were planted in the pots, rhizomes related to salt stress and salt stress treated by nano alloy Au-Ag were sprayed with sodium chloride solution for 20 days every other day (the whole body of the plant would get wet). After the end of the treatment period, the plants of the control and treatment groups were collected for measurements. In each pot, there were seven peppermint peppers. To measure the dry weight of the samples, they were placed in an oven at 70°C for 72 hours, and wet samples were stored in a freezer at -80°C for further testing (Prasad *et al*., 2012).

For the length of root and shoot measurement, the length of the longest root from the collar to the tip of the root and the length of the longest stem from the collar to the tip of the meristem were measured using the ruler of the caliper and for the fresh weight of roots and leaves measurement, after the growth period, the components of each pot (containing root, stem, and leaves) were separated and washed with distilled water. After drying the samples, their fresh weight was measured by a digital scale with a precision of 0.001.

Chlorophyll and carotenoid measurements were done by Lichtenthaler and Wellburn methods (Lichtenthaler and Wellburn, 1983). 0.1 gr of mint leaves was crushed in the Chinese moss in the presence of 5 ml acetone (80%). The extract was centrifuged for about 10 mins. at a rate of 2500 rpm. Then, the upper phase was separated and absorbed by spectrophotometer (UV-vis, CECL 5505) at 663,645 and 470 nm wavelengths. The content of chlorophyll and carotenoids are calculated from the following formulas:

Chlorophyll a (mg/g) = [12.7 (A663) - 2.69 (A645)] ×V/1000×W

Chlorophyll b (mg/g) = [22.9 A645) - 4.68 (A663)] ×V/1000×W

Total chlorophyll (mg/g) = [20.2 (A645) + 8.02 (A663)] ×V/1000×W

Carotenoid (mg/g) [A480 + (0.114 A663) - (0.638 A645)] ×V/1000×W

V: final volume of chlorophyll extracted in 80% acetone; W: fresh weight of the *Mentha* 's powder used

Anthocyanin Measurements were done by (Wagner, 1979) methods:0.1 gr of mint leaves was crushed in the 10 ml of acid solvent (99% methanol, 1% acid chloridric). Then, extracts were centrifuged for about 20 mins. at 3000 rpm .The upper phase was separated and stored for 24 hrs. in darkness at room temperature. Then, the absorption of each sample was measured at

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550 nm by the spectrometer.

The method of Bates was used to measure the amount of proline free (Bates *et al,*. 1973). 4 gr of sample leaves were washed in the presence of 15 ml sulfosalicylic acid (3%) and left it for 3 hrs. at 4°C for extraction to complete. After 72 hours the samples were centrifuged for 20 minutes at 3000 rpm. Then, add 2 ml of the aqueous solution of 2 ml of glacial acetic acid and 2 ml of reagent ninhydrin (containing 20 ml of phosphoric acid of 6 m, 30 ml of glacial acetic acid, and 25.1 g of ninhydrin). The samples were boiled in a water bath at 100°C for 1 hour. After removing the specimens from the hot water bath, the samples were cooled rapidly by placing in an ice path (stop the reaction). and on each sample, 4 ml of toluene was added and mix vigorously. After the formation of two phases, the absorbance of the supernatant for each sample at 520 nm was read by the spectrophotometer. To determine the proline content of the samples, the standard curve was prepared using proline specific concentrations. At 4°C, the reagent is stable for 24 hrs. A standard curve was used for concentration from 0-512 µL (20-100 µg/ml) of L-proline.

The amount of soluble sugars in phenol sulfuric acid is based on the acidic hydrolysis of soluble sugars and the formation of a phosphorus compound that produces a complex with phenol producing a colored complex (Dey, 1990). 0.5 g of fresh weight of the plant was weighed from each treatment and was thoroughly crushed in 5 ml of distilled water. The resulting extract was taken 2 ml and transferred to the test tube and 9 ml of 5% phenol was added to each of the test tubes. After 1 hrs., the absorbance was measured at 485 nm by a spectrophotometer. The sugar content of the solutions was measured from the absorbance conformance of the samples with the standard curve .

Preparation of plant extracts for determination of catalase (CAT) and guaiac peroxidase (GPX) activity was carried out based on Aebi (Aebi, 1984) and Upadhayaya protocol (Upadhyaya *et al*., 1985) with slight modifications. 0.5 g of leaves were placed into a cold mortar/pestle. Enzyme extraction was done with 3 ml of Tris buffer (pH = 7.5) that contained 3 mM and 1 mM EDTA (and extraction buffer for measuring the activity of APX has consisted of 0.2 mM ascorbate). The homogenates were centrifuged for 20 mins. at 4°C at 5000 rpm. The supernatant was used as a crude extract for measuring the activity of antioxidant enzymes. This method was continuous spectrophotometric rate determination. One unit of catalase will decompose 1.0 µmole of H_2O_2 per minute at pH 7.0, while the rate of disappearance of H_2O_2 is observed as a rate of decrease in absorbance at 240 nm.

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Reagent preparation:

1) Phosphate buffer (50 mM) pH 7.0 (889 mg of Na_2HPO_4 in 100 ml)

2) H_2O_2 (30 mM): 30% H_2O_2 was prepared and 340 µl of this 30% H_2O_2 was added to phosphate buffer and the volume made up to 100 ml with the phosphate buffer (It was prepared fresh).

Procedure: 1:500 (10 ul in 5 ml) plant extract was made with phosphate buffer. And the reagents were added in the following sequence: A decrease in absorbance was recorded from 0-60 sec. at 240 nm

$$\left[\frac{\Delta \text{Absorbance / min}}{EC} \times \frac{TV}{SV} \times DF\right] \Big/ \text{Hb} \times 10$$

EC (extinction coefficient) = 0.0436, TV (total reaction volume) = 1010 µl, SV (sample volume) = 10 µl, DF (dilution factor) = 501X2

The measurement of GPX activity was performed by the Upadhyaya method (Upadhyaya, 1985). 3.5 ml phosphate buffer of 50 mM (pH 7.0) contained 1 ml of 1% guaiacol, 1 ml of hydrogen peroxide 1%, and 0.1 ml of extracts were prepared. GPX activity was calculated as an increase in absorbance over 1 minute at 240 nm. To measure activity, the offset factor (6/26 Mm^{-1} cm^{-1}) and the following formula were used.

$$\left[\frac{\Delta \text{Absorbance / min}}{EC} \times \frac{TV}{SV} \times DF\right] \Big/ \text{Hb} \times 10$$

EC (extinction coefficient) = 26.6, TV (total reaction volume) = 1010 µl, SV (sample volume) = 10 µl, DF (dilution factor) = 501X2

Statistical analysis: The results were analyzed by comparing (F) values obtained from an ANOVA using the SPSS statistical package (Gerber *et al*., 1997). The lowest significant (LSD) between the means, at the (5%) level, was determined for various treatments, following the method of Steel and Torrie, (1981). In all diagrams, the vertical columns indicate the mean and standard deviation for three repetitions.

Results

Au and Ag's ions are reduced as soon as they are exposed to mentha extracts, forming an Au-Ag nano-alloy that changes the color of the solution from colorless to reddish-brown. The formation of nanoparticles can be investigated by UV-Vis spectroscopy, TEM, and DLS imaging methods. Given the following figure and comparing the data with the results of chemically synthesized nanoparticles, it is clear that the extract can form Au-Ag nanoparticles, so that the intensity of the absorption spectra at wavelengths of 510 nm is significant (Figure 1 a and b).

As can be seen in figure 2, under salinity stress conditions, the effects of Au-Ag alloy nanoparticles and different salinity concentrations have caused variety in physiological factors .

Variance analysis shows that the effects of salinity,

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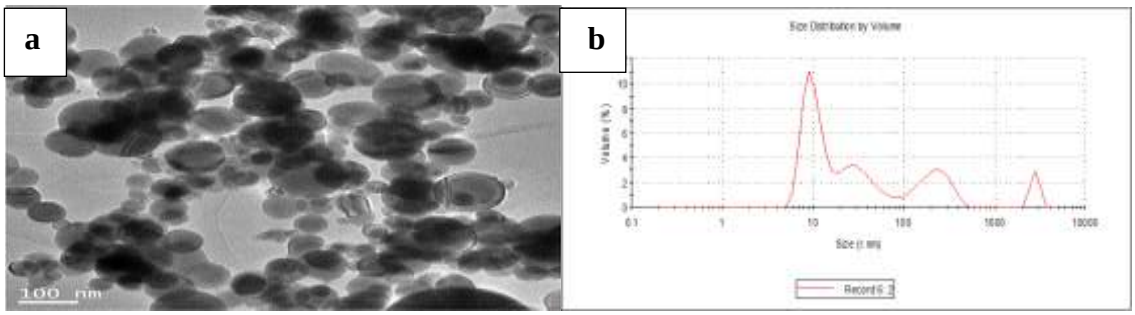


Figure 1. TEM image (a) and DLS image (b) of Au-Ag nano alloy formed by *Mentha Piperita* 's extract



Figure 2. Effects of Au-Ag alloy nanoparticles and different salinity concentrations on physiological factors of *Mentha piperita* under salinity stress conditions (A: Control B: Ag/Au alloy NPs C: Ag Nitrate D: Au Chloride E: NaCl 50 mM F: NaCl 50 mM + Ag/Au alloy NPs G: NaCl 100 mM H: NaCl 100 mM + Ag/Au alloy NPs I: NaCl 150 mM J: NaCl 150 mM + Ag/Au alloy NPs)

Table 1. Results of variance analysis for physiological and morphological traits of *Mentha piperita*

Source of variation	df	MS								
		length of root	length of shoot	fresh weight of root	fresh weight of shoot	Chl a	Chl b	carotenoid	anthocyanin of root	anthocyanin of shoot
salinity (s)	3	142.30**	122.26**	0.88**	2.01**	0.324**	0.591**	0.511**	0.68**	0.13**
nanoparticle(n)	3	292.50**	41.37**	0.85**	10.48**	0.468**	0.765^{ns}	0.668**	0.96**	0.24**
(s) × (n)	9	81.65**	16.50**	0.27**	1.19**	0.081**	0.099**	0.079**	0.15**	0.12**
Error	30	21.61	0.49	0.6	0.38	0.009	0.030	0.004	0.16	0.18
CV (%)		7.08	3.87	24.32	8.12	13.29	25.17	12.35	3.35	9.68

**** significant at 5% and ns significant shoot lengths**

Continue of table 1.

Source of variation	df	MS							
		soluble sugar of root	soluble sugar of shoot	proline of root	proline of shoot	CAT of root	CAT of shoot	CPX of root	CPX of shoot
salinity (s)	3	0.07**	0.26**	0.22**	0.01**	0.024**	0.003**	0.93**	3.01**
nanoparticle(n)	3	0.53^{ns}	0.37**	0.27**	0.48**	0.068**	0.001**	0.91**	11.49**
(s) × (n)	9	0.045**	0.050**	0.12**	0.19**	0.081**	0.004**	0.33**	2.91**
Error	30	0.009	0.049	0.036	0.038	0.009	0.004	0.10	0.56
CV (%)		6.2	3.87	16.8	18.12	19.29	15.7	28.36	9.84

**** significant at 5% and ns significant shoot lengths**

of Au-Ag nanoparticles, and the interaction of both of them on the total of measured factors (except the effects of Au-Ag nanoparticles on the soluble sugars content) are statistically significant (Table 1).

The figure 3 shows the effects of various salinity concentrations in the presence and absence of nano alloy on the length of the peppermint's roots and shoot. As can be seen, rising salt concentration caused a significant decrease in root and shoot length. Also, the positive effect of treatment with Au-Ag nano-alloy solutions reduces the effects of stress on root and shoot lengths.

The effect of nanoparticles and different salinity concentrations on root and shoot fresh weight of peppermint (Figure 4) has followed its effect on the fresh weight of root and leaf of the plant .

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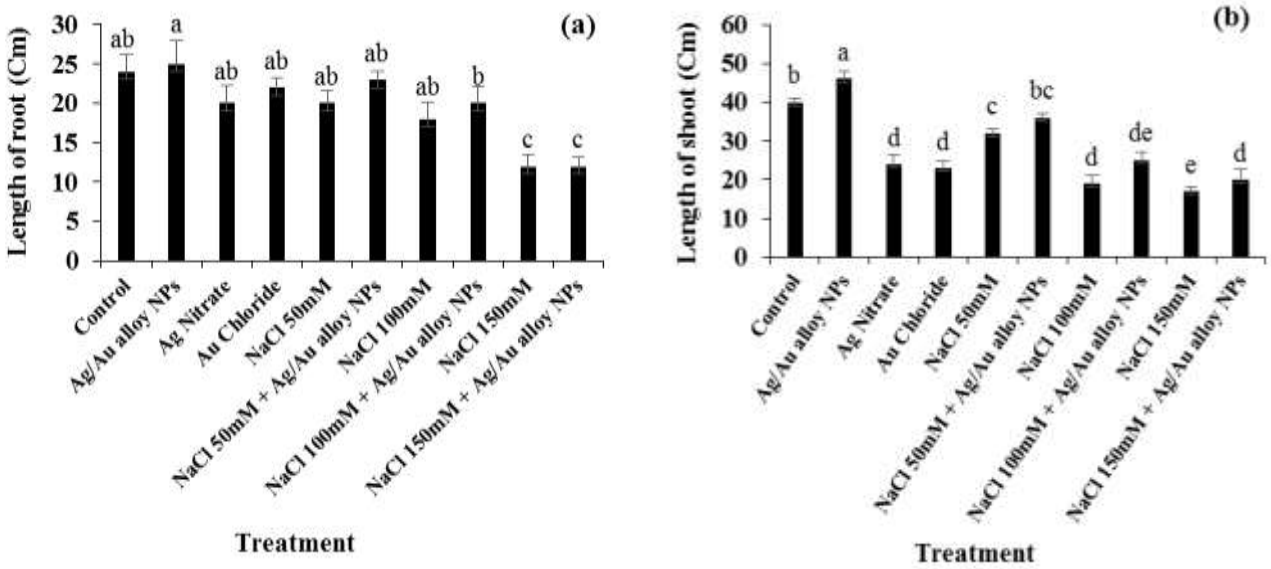


Figure 3. The effect of various salinity concentrations in the presence and absence of nano alloy on length of *Mentha Piperita* '*s* roots (a) and shoot (b). Means with the same letters are not significantly different at 5% (Duncan) probability level

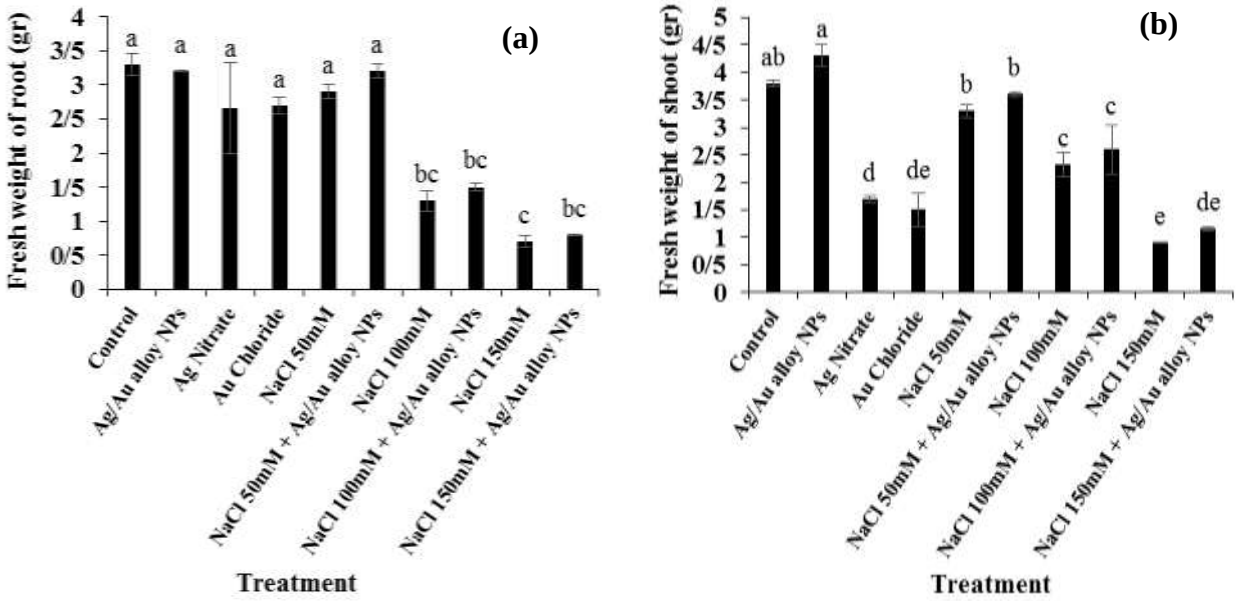


Figure 4. The effect of various salinity concentrations in the presence and absence of nano alloy on the root (a) and shoot (b) fresh weight of *Mentha Piperita.* Means with the same letters are not significantly different at 5% (Duncan) probability level

According to figure 5, salinity decreased the chlorophyll a and b levels in all plants under salt stress, $AgNO_3$, and $AuCl_3$. This is while nano alloy Au-Ag treatment significantly increased levels of chlorophyll a and b at a 5% probability level in these plants. The results of the studied the total carotenoid in the leaf of control plants and treated with nano alloy Au-Ag and salinity (Figure 5) show that salinity stress had a significant effect on the carotenoid level at 5% probability level while nano alloy Au-Ag treatment did not show a significant effect on the carotenoid activity of the plant under salt stress. According to figure 5, anthocyanin levels in plants under salt stress decreased significantly. However, nanoparticles did not affect the improvement of stress conditions on plant growth. The highest amount of anthocyanin was related to the control sample and the lowest amount is related to the sample under salinity stress of 150 mM NaCl.

Figure 6 shows with the increase in the content of salt concentration, the proline content of the plant increases significantly. Also, under treatment with nanoparticles, the amount of proline in the leaves of treated plants decreased compared to the control, while the amount of proline in the root did not show a significant change compared to the control. In samples treated with salt and silver nanoparticles, the nanosilver solution reduced the proline content of plant leaves and roots. The highest amount of proline in the leaves and roots of peppermint plants was related to plants under the stress of 150 ml of sodium chloride and the lowest

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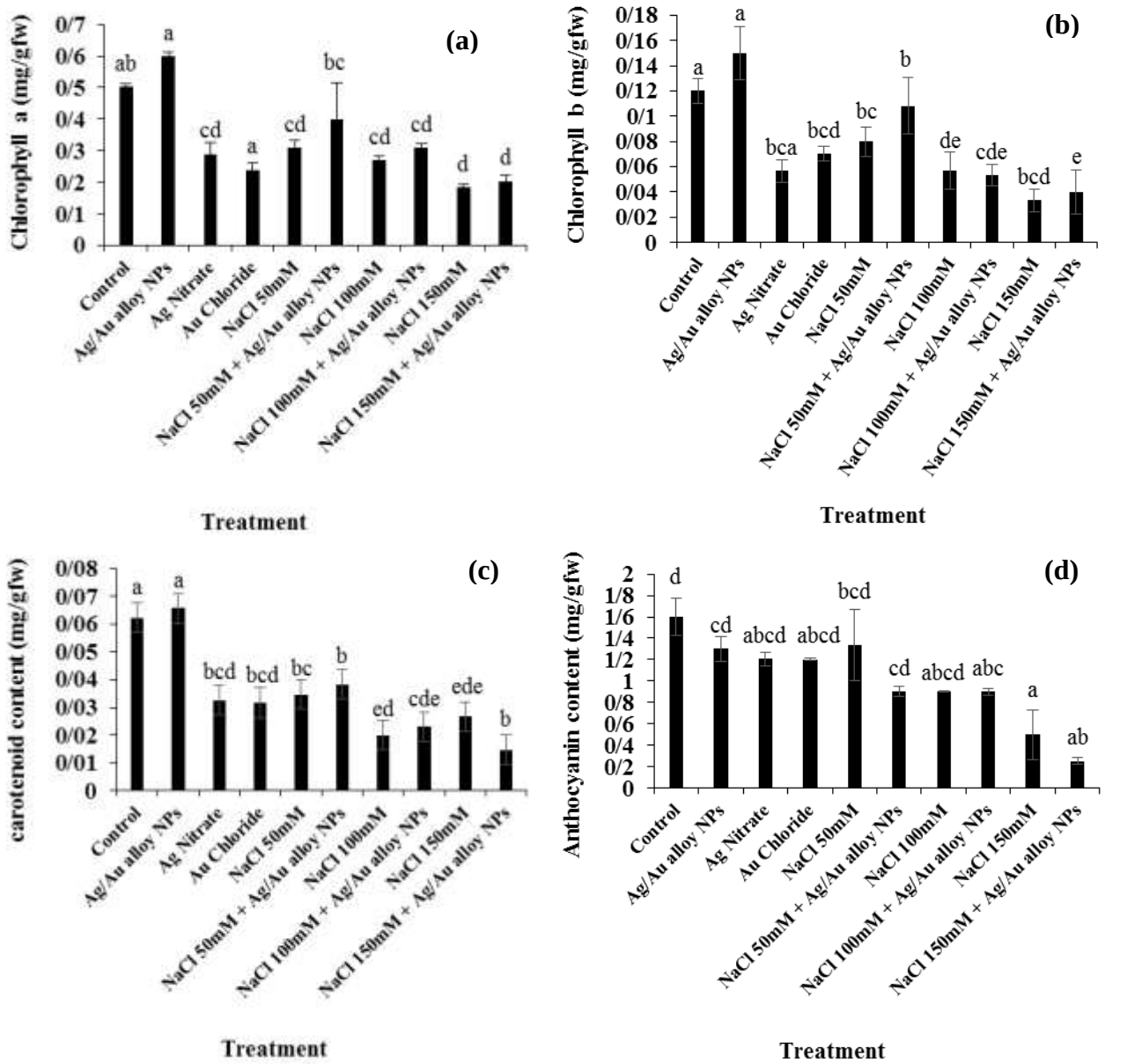


Figure 5. The effect of nano alloy Au-Ag and different salinity concentrations on *Mentha Piperita* 's chlorophyll an (a) and chlorophyll b (b) and carotenoid (c) and anthocyanin (d) content. Means with the same letters are not significantly different at 5% (Duncan) probability level

amount was related to the samples sprayed with Au-Ag nano-alloy.

Study of the effects of salinity and nano alloy Au-Ag on the content of different kinds of soluble sugar showed that increasing the salinity concentration significantly increased the soluble sugar content of the plants and this effect was reduced in the presence of nano alloy Au-Ag (Figure 7). However, the amount of sugar soluble in plants under the treatment of silver nitrate and gold chloride compared to control plants has shown a significant increase. Also, the amount of soluble sugars in plants under salinity stress with nanoparticles showed a significant decrease compared to plants under salinity stress. The highest levels of leaf and root soluble sugars were observed in plants under the stress of 150 mM sodium chloride and the lowest in plants sprayed with Au-Ag nanoparticles.

Figure 8 shows the activity of a catalase enzyme under salinity stress and nano alloy Au-Ag in *Mentha piperita*. Along with increasing salinity at the culture medium, the activity of catalase enzyme increased significantly. However, the treatment of Au-Ag nano-alloy under salinity stress significantly reduced the activity of the catalase enzyme. Also, silver nitrate solutions and gold chloride significantly reduced enzyme activity compared to the control. The highest activity of catalase enzyme was observed in plants under the stress of 150 ml of sodium chloride and the lowest in plants treated with nanoparticles.

Figure 9 shows the changes in the activity of the

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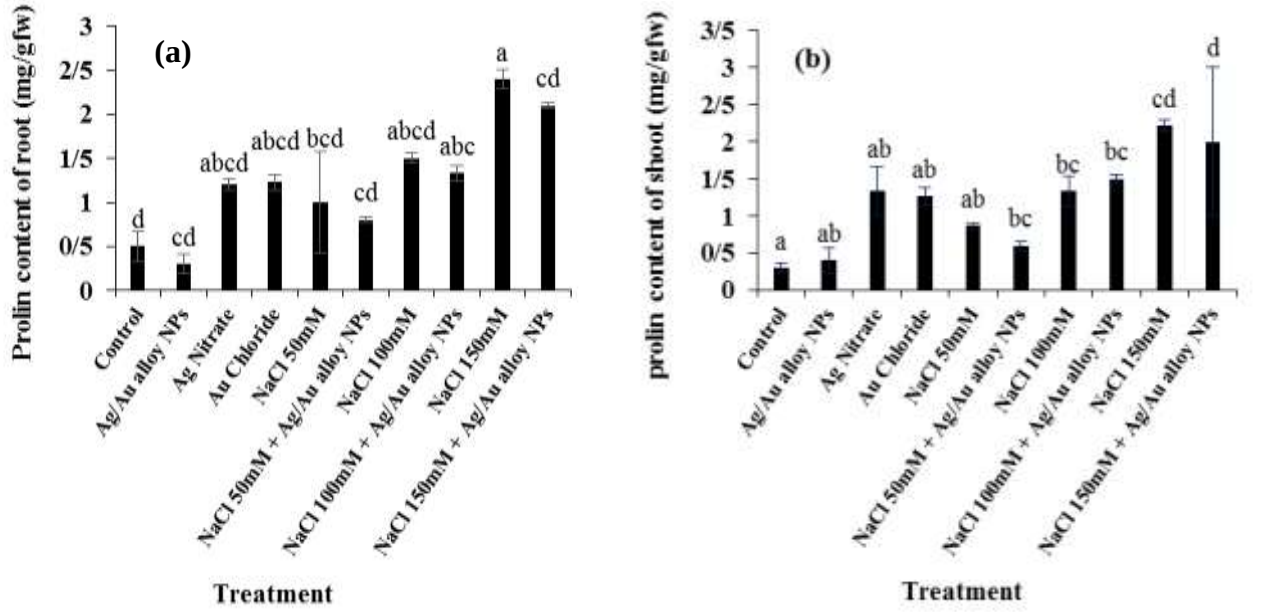


Figure 6. The effect of nano alloy Au/Ag and different salinity concentrations on the Proline content of *Mentha Piperita* 's root (a) and shoot (b). Means with the same letters are not significantly different at 5% (Duncan) probability level

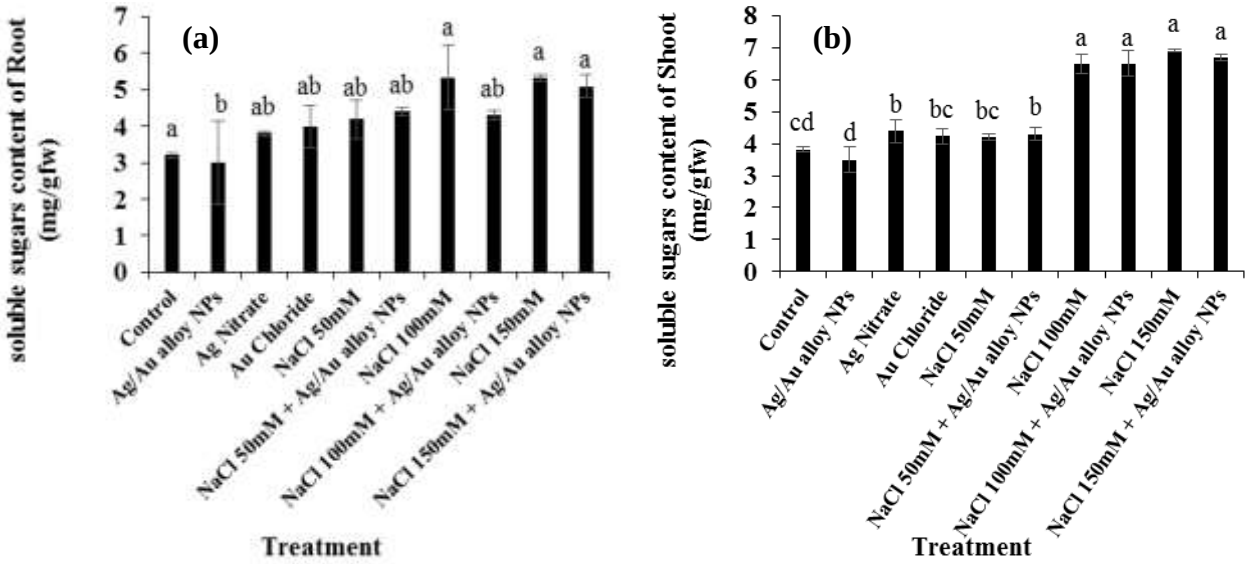


Figure 7. The effect of nano alloy Au/Ag and different salinity concentrations on soluble sugars content of *Mentha Piperita* 's root (a) and shoot (b). Means with the same letters are not significantly different at 5% (Duncan) probability level

enzyme guaiac peroxidase under salinity stress and the nano alloy Au-Ag in peppermint herb. Increasing the concentration of salinity in the medium increased the activity of the catalase enzyme. The treatment of nano alloy Au-Ag under salt stress conditions could moderate the negative effects of salinity stress. The highest activity of GPX enzyme was observed in plants under the stress of 150 ml of sodium chloride and the lowest in plants treated with nanoparticles.

Discussion

When the plant is under salinity stress, all important processes, such as photosynthesis, protein synthesis, and metabolism, energy, and lipid are affected. In this study, the general examination of the apparent symptoms in peppermint plants under the treatment of nano alloy Au-Ag, $AgNO_3$, $AuCl_3$, and various salinity concentrations showed that increasing salinity reduced the growth of the plant and also reduced the growth parameters in the plant. However, the presence of nanoparticles and salinity stress can significantly improve these negative effects on plant growth parameters. These findings are supported by many studies (Sudhakar *et al.*, 2012). According to the findings of Bandeoglu *et al*. (2004), morphologically, the most significant symptom of salinity damage to the plant is low growth due to the inhibition of cell proliferation (Bandeoglu *et al*, 2004). Parvaiz Ahmad and his colleagues have reported that

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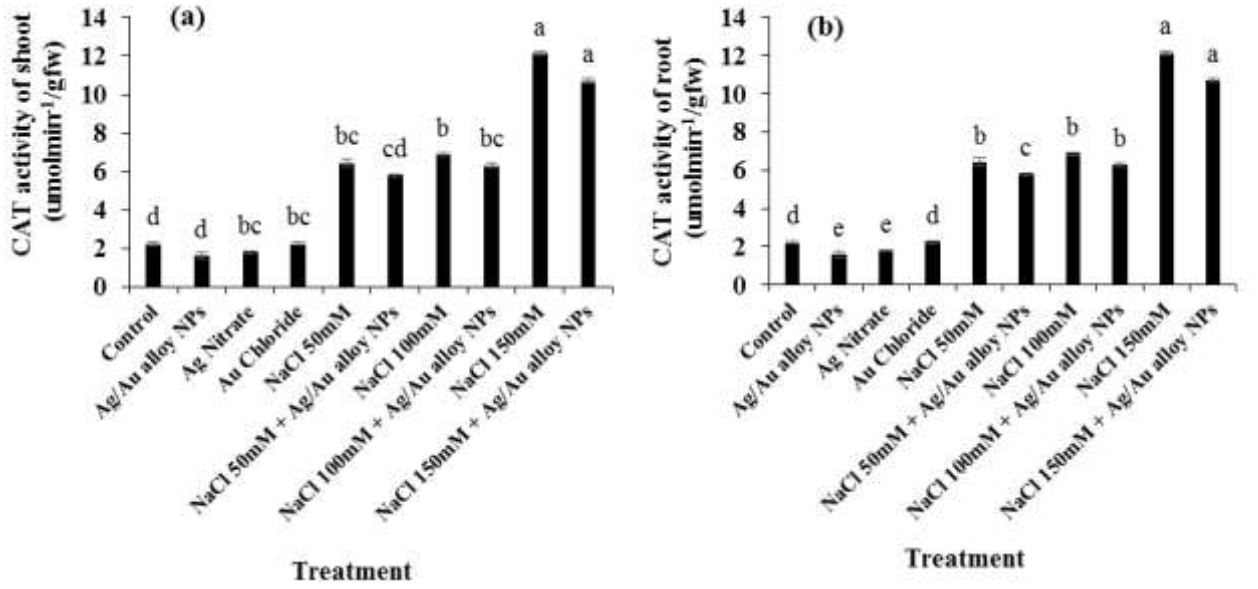


Figure 8. The effect of nano alloy Au/Ag and different salinity concentrations on the activity of catalase of *Mentha Piperita* 's root (a) and shoot (b). Means with the same letters are not significantly different at 5% (Duncan) probability level

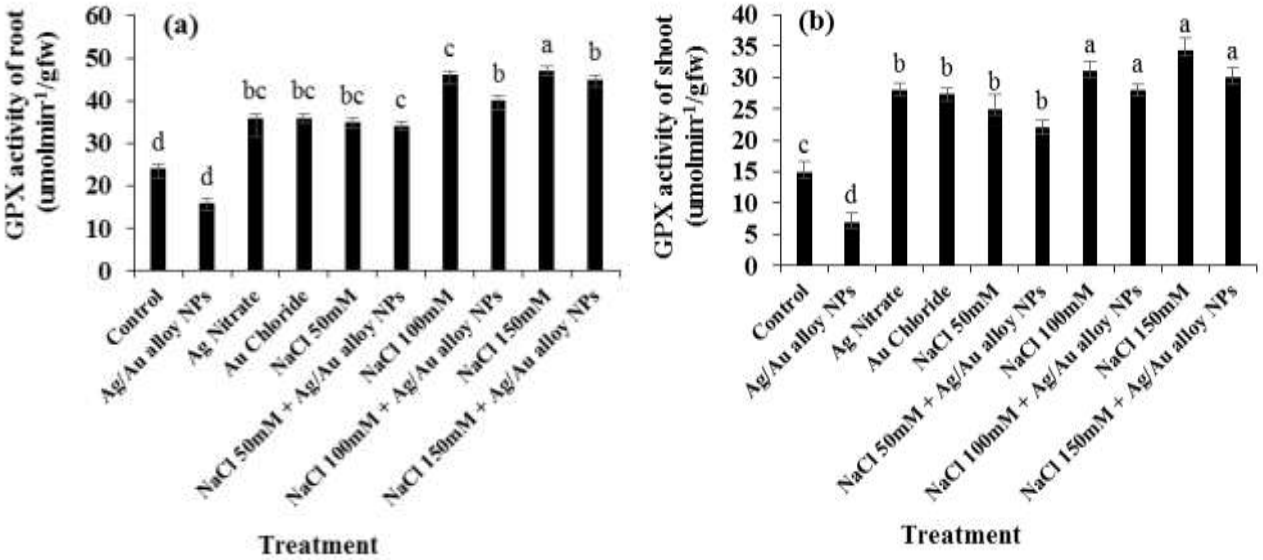


Figure 9. The effect of nano alloy Au/Ag and different salinity concentrations on the activity of the enzyme guaiac peroxidase of *Mentha Piperita* 's root (a) and shoot (b). Means with the same letters are not significantly different at 5% (Duncan) probability level

salt and ion accumulation causes osmotic stress and dryness. It results in a decrease in water absorption of plant tissues, resulting in a decrease in the water content of the tissue and the reduction of root growth and cell development. Thus, the reduction of water absorption and its implications for cell growth is one of the most important reasons for decreasing root and stem growth (Parvaiz Ahmad *et al*., 2016).

Salinity disrupts cell division as well as cell growth, and all metabolic reactions of the plant are affected. The excessive amount of sodium and chloride ions reduces the absorption of essential ions, such as potassium, calcium, ammonium, and nitrate. It also reduces enzyme activity and disrupts the structure of the membrane. These effects reduce the plant's metabolic activity, including photosynthesis, and reduce plant growth in saline conditions (yin *et al*., 2011). Also, it reduces and retards germination, decreases the growth of the airways, and reduces the production of dry matter (Rizwan *et al*., 2015). Reduction in the biomass increases with increasing salinity, which is apparently due to the degradation of biochemical and physiological activities, under salty conditions, which may be due to the decrease in the leaf area and the number of leaves. It seems that decreasing leaf area and other plant organs due to increased salinity due to the decrease in the level of growth hormones such as auxin, glycolic acid, and cytokinin, and an increase in growth inhibitor such as

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acid abnormalities (Iqbal and Ashraf, 2007). The results of Pandya *et al*.'s research on barley plants affected simultaneously with sodium chloride stress and spray application of 50 ppm silver nanoparticles showed an improvement in plant growth parameters (Pandaya *et al*., 2005). This result is similar to Najafi's research on the wheat seed. He showed that treatment of silver nanoparticles with a concentration of 50 ppm increased germination percentage, increased stem length and root, and finally improve the establishment of wheat (Karami *et al*., 2016). Interestingly, nanoparticles increase plant growth parameters under stress conditions. While $AgNO_3$ and $AuCl_3$ are the main components of the synthesis of nanoparticles, they are the factors that reduce plant growth (Najafi *et al.*, 2013).

The results of this study showed that salinity stress significantly decreased dry weight, and treatment of nano alloy Au-Ag caused fresh and dry weight gain in peppermint plants. Juan and his colleagues have shown in their studies that the treatment of silver nanoparticles would increase plant weight, increase root length, plant height, and several plant stems. It seems that the application of these silver nanoparticles can reduce the effects of drowning stress such as weight loss, altitude reduction, etc. (Juan *et al.*, 2005). One of the important physiological factors in plant growth is its chlorophyll content. Chlorophyll is bound to the membrane and depends on the stability of the membrane, which remains rarely healthy under salt stress. Chlorophyll is characterized by having centralized magnesium with a quaternary isocyclic loop, derived from the propionic acid group, at the position of six precursors of porphyrin from other tetrapyrrole molecules. In the present study, an increase in salt concentration has reduced the amount of chlorophyll a and b. A decrease in the content of chlorophyll can be due to the increased activity of chlorophyll degrading enzymes, chlorophylls under stress conditions (Noreen and Ashraf, 2009). High accumulation of sodium in plant tissues has been reported as an effective factor in the growth of photosynthetic pigmentation and photosynthesis rate (Iqbal and Ashraf, 2007). Another study has suggested that the reduction of photosynthetic pigmentation in environmental stresses depends on the genotype of the plant (Juan *et al*., 2005). Generally, the reduction of the synthesis of the major complex of chlorophyll pigment, the intensification of the activity of chlorophyllase and peroxidase enzymes, the production of phenolic compounds, the increase of reactive oxygen species and damage to the chloroplast membrane, and disruption of nitrogen absorption of soil are known as the most important factors reducing the concentration of chlorophyll in severe stress.

Proline plays an important role in modulating the osmotic pressure of the cell under stresses such as salinity, low temperature, food shortages, exposure to heavy metals, some chemical compounds, high acidity, and other stresses. Proline reduces the effect of ions on enzymes and enhances the stability of enzymes at high temperatures. Other proline functions in the plant include the carbon and nitrogen reserve for the tissues under repair, an effective combination of regulating osmotic pressure, a buffer for pH stabilization, a cleansing agent for oxygen reactive cells, and the title of a protective molecule (Ghanati and Bakhtiarian, 2013).

In the present study, an increase in sugar content in leaves and roots was observed after increasing salinity stress. The accumulation of sugar in the organs of the plant and the reduction of starch in them, which is achieved by the destruction of large molecules in the cells of the plants to avoid plasmolysis and the establishment of thoroughness due to environmental stresses, resulting in coarser molecules such as starch to sucrose and then glucose and fructose break down, resulting in a negative potential for water in the cells and osmotic regulation. In addition to converting starch into soluble sugar, reducing sugar intake is another factor in increasing sugar in the cell. Researchers have also reported that glucose increase in saline plants has occured due to the effect of this stress on reducing the power transmission capacity of the aquifer or reducing the consumption of consumer organs. Sugar is also associated with increased activity of enzymes such as phosphoryl starch, sucrose phosphate, and invertase activity (Dubey and Singh, 1999).

Peroxides and catalases are two major systems for enzymatic defense and peroxidative damage to cell walls that are controlled by the ability of the antioxidant enzymatic system. Gaiacol peroxidase uses oxidation of phenolic compounds such as guaiacol to detoxify and decompose hydrogen peroxide (H_2O_2) and is also found in the cytosol, cell walls, and vacuoles. Hydrogen peroxide is a toxic substance caused by many of the cell's natural mechanisms and reactions. Accumulation of this substance is highly damaging to cells and tissues and should be decomposed immediately. Phenolic compounds such as guaiacol act as electron donors to hydrogen peroxide. Catalase is another effective defense enzyme that is produced in all living organisms under stress. This enzyme reduces its toxic effects by having a direct effect on hydrogen peroxide. Catalase uses hydrogen peroxide as a substrate and rapidly degrades it to inhibit its destructive effects. It is present in cytosol, mitochondria, chloroplasts, as well as peroxisomes.

In the present study, an increase in the concentration of antioxidant enzymes including catalase and guaiac peroxidase was observed with increasing salinity concentration in the medium. Salt stresses increase the amount of MDA and dehydrogen peroxide in the plant, which leads to degradation of macromolecules and induces membranes in the plant. In this condition, the plant is involved in an enzymatic and non-enzymatic antioxidant defense mechanism to overcome oxidative stress. In a study on tomato plants, the silver nanoparticles reduced the activity of the catalase and superoxidase enzymes in the leaves and roots of the plant up to a concentration of 75 mg and at higher concentrations. These observations indicate that the

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effect of nano in the antioxidant enzyme varies with plant species, dose, and nano time. Reductions in the amount of catalase and peroxidase enzymes under the nanoscale are probably due to lower production of MDA and hydrogen peroxide in the leaves than the control. The non-enzymatic defense system in plants contains antioxidant compounds such as anthocyanins, carotenoids, tocopherols, ascorbic acid, and phenolic compounds. Anthocyanin flavonoids are one of the most antioxidant compounds. These compounds not only eliminate free radicals but also prevent further production of the plant. Anthocyanins are more likely to facilitate the entry of salt into the vacuole of the cells and, as a result, collect them from other parts. Research shows that anthocyanins can act in harmony with protective molecules in plant cells and act to compensate for violations of the concentration of molecules during the stress period. Anthocyanins can enter in special places within the leaves for optimal plant performance. The accumulation of anthocyanins is induced by various stimulants such as UV, low temperatures, pathogens, and several growth regulators such as cytokinin, gibberellins, ethylene, and salicylic acid (Gould and Lister, 2006). These observations are probably due to the role of anthocyanin in quantitative and qualitative adjustments of light absorbed, protecting optical control, and sweeping the active oxygen species under environmental stress. The amount of anthocyanin was decreased under salt stress conditions with Au-Ag nano-alloy treatment. This is probably due to the presence of anthocyanin precursor in another route of synthesis of materials or because of the reduction of oxidative stress in these treatments by inhibiting the effects of ethylene by nanoparticles. (Yang *et al.*, 2008).

Conclusion

Salinity stress was studied as a limiting factor for plant growth factors and its effect on reducing plant growth was proven. Meanwhile, nanoparticles were introduced as factors that improve plant resistance in response to salinity stress, and their effect was proven. The results could be an important step in cultivating salt-resistant peppermint species in areas facing this type of stress.

Acknowledgments

The authors would like to acknowledge the faculty of Payam-Noor Tehran for their support and contribution to this study and we also thank Dr.Chaichi (Department of Chemistry, University of Mazandaran, Babolsar, Iran) for providing us technical supports.

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