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Investigation of Physiological and Biochemical Responses and Essential Oil Yield of Peppermint Under Salt Stress

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Peppermint (Mentha piperita L.) is an economically important medicinal and aromatic plant grown in different areas worldwide. Secondary metabolites were fundamentally produced by genetic processing; however, environmental factors affect their biosynthesis. Salinity is the most important abiotic stress which induces morphological, physiological, and biochemical changes in plants. To investigate the influence of salinity stress (0, 25, 50, 75, 100 and 125 mM NaCl) on chlorophyll content, stomatal conductance, relative water content (RWC), proline, Na+ and K+ content, antioxidant enzymes of catalase (CAT), peroxidase (POX) and polyphenol oxidase (PPO), protein, essential oil yield and dry weight of peppermint, a greenhouse study was conducted. The results indicated that salinity had a significant effect on foregoing parameters. Changes in chlorophyll content were peak and stomata conductivity was a single function. Based on estimations, the highest chlorophyll content was recorded for low salinity (60 mM NaCl). The plant proline content was higher in stress condition compared to control plants. The highest proline content observed in 125mM NaCl concentration was two times higher than that of control plants. There was an increase and then decrease in CAT and POX activities, respectively, in lower and sever levels of salinity. A single equation was the best-fit equation for changing PPO enzyme activity under stress conditions. The dry matter has been affected dramatically by salinity and decreased from 11.34g under the non-stress condition to 4.24 g under high stress condition. Essential oil percentage (in dry matter) increased in moderate salinity stress. We found that the amount of essential oil per plant was linearly decreased. So, the highest (9.78 g plant-1) amount of essential oil per plant belonged to control group and the lowest (4.6 g plant-1) was observed for full stress condition.

Keywords: Peppermint, Essential oil, Salt Stress, Proline, chlorophyll, Enzyme.

One of the economically important medicinal and aromatic plants grown in different areas in the world is Peppermint (Mentha piperita L.) (Yazdani *et al*., 2002). The plant's leaves have traditionally been used as a spice, and its essential oil is widely used in food, cleaning, pharmaceutical, and cosmetic industries as it is both flavoring and fragrant (Herro and Jacob, 2010). Menthol, menthone, methylacetat, menthofuran and pulegone are among fundamental components of peppermint essential oil (Tabatabaie and Nazari, 2007; Mahmoud and Croteau, 2003).

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Genetic processing is the basic mechanism through which secondary metabolites are produced in the medicinal and aromatic plants; however, environmental factors strongly affect their biosynthesis (Aydan *et al*., 2002(. Biotic and abiotic environmental factors affect growth parameters, essential oil yield, and ingredients (Aziz *et al*., 2008; Clark and Menary, 2008). Particularly, salt stress can significantly affect plants' morphology, physiology, and biochemistry (Khorasaninejad *et al*., 2010; Queslati *et al*., 2010). Salinity may result in > 60% decrease in crops' potential yield (Munns, 2002; Tuteja *et al*., 2013).

High amounts of salt in the environment will disrupt the homeostatic balance of water potential and ion distribution within a plant. Sodium toxicity may elicit a wide array of disorders that affect development, germination, protein synthesis, photosynthesis, lipid metabolism, leaf chlorosis, and senescence (Santhi *et al*., 2013). Salinity induces oxidative stress in plant through increased production of reactive oxygen species (ROS). Plants have developed complicated defense mechanisms against oxidative stress, which includes regulation of antioxidant enzymes activity, scavenging ROS, and adjusting ionic and osmotic levels (Ashraf and Harris, 2004). Antioxidant enzymes such as peroxidase (POX), superoxide dismutase (SOD), and catalase (CAT) play an important role in defense against oxidative stress (Alscher *et al*., 2002).

To minimize ionic toxicity, plants establish ionic compartments within cells or excrete ions. They also increase cellular levels of somatically compatible solutes, such as proline, to maintain ionic homeostasis and stabilize proteins in saline conditions (Oo *et al*., 2015; Lim *et al*., 2015; Ibrahim *et al*., 2015). Although the effects of salinity on traditional crops have been the subject of extensive research, that is not the case for medicinal plants (Aghaei and Komatsu, 2013). Santhi *et al*. (2013) showed that biosynthesis of secondary metabolites is affected strongly by salt stress, resulting in considerable fluctuations in essential oil quality and quantity. Aziz *et al*. (2013) reported that essential oil under salt stress was reduced in Peppermint, Pennyroyal and apple mint, as compared with untreated control group which was not under salt stress. In a research carried out by Roodbari *et al*. (2013), it was found that an increasing in the salinity lead to reduction in length of shoot and root, fresh weight of stem and root, dry weight of stem and root, internodes length, total biomass and essential oil percent in Peppermint. The highest values of growth parameters and essential oil percentage were observed under non-salinity condition (Roodbari *et al*., 2013). Ozkan and Baydar (2016) concluded that salinity deceases the content of essential oil, but increases lipid peroxidation, proline, and antioxidant enzyme activities. Xu *et al*. (2016) reported that salinity resulted in increases in malondialdehyde and proline contents in the aerial parts and roots of peppermint and thistle, although chlorophyll content was not affected. They also found a decrease in potassium (K+)/sodium (Na+) and total soluble protein content in the aerial parts and roots in both species. This present research was designed to understand the effects of salinity stress on biochemical and physiological characteristics of peppermint and also on the synthesis of essential oil in this plant. Investigating of peppermint tolerance and physiological responses to different salinity levels was another object of this paper.

METHOD AND MATERIAL

Plant material and growth condition

This was a greenhouse experiment with a randomized complete block design. Experimental treatments involved six levels of salinity stress made by pure NaCl at 0, 25, 50, 75, 100 and 125 mM concentrations.

Each pot was filled with 0.5 kg perlite and nutrient with Hoagland's solution. Chemical characteristics of the Hoagland's solution are shown in Table 1 (Noroozi, 2001). Two-week seedlings with 3-4 true leaves were transferred to pot. The nutrient solution was aerated continuously and replaced every two days. After 20 days, for the salt treatments, soluble NaCl was added to the culture medium until the salt concentrations reached the final level.

Assay of chlorophyll and stomatal conductance

The total chlorophyll content was determined by using a chlorophyll-meter device (SPAD model 502, Minolta, Japan) after applying the treatments and before harvesting. In such a way, the measurementwas carried out from the bottom, middle, and upper parts of each plant.

Stomatal conductance was recorded by Leaf porometer device (eijkelkamps model, Netherlands). Clamps of device attached to the leaves and the continuous air flow through the stomata was measured.

Assay of osmotic adjustment parameters

We used 2 leaves (detached from same position) per plant to determine relative water content (RWC). The leaves were cut and the petioles were immediately immersed in distilled water in pre-weighed glass tubes. The tubes were sealed and taken to the laboratory and weighed; the new weight of each tube was used to determine leaf fresh weight (FW). The leaves were left for 48 h in dim light and were weighed again to measure their turgid weight (TW). Then, dry weight (DW) was determined after oven-drying at 80æ%C for 48 h, and RWC was calculated as follows:

RWC = 100 × (FW - DW)/(TW - DW)

Proline content was determined spectrophotometrically using ninhydrin with the adopted mixture method from Iqbal *et al*. (2015). Fresh leaf tissues (0.1 mg) were homogenized in 2 mL of 3% sulphosalicylic acid. Then centrifuged with 4000 rpm for 10 min at room temperature. 1mL of acid ninhydrin and 1mL of glacial acetic acid was added to 1mL of supernatant and the reaction was carried for 1 h in a test tube placed in a water bath at 100æ%C. Finally, 2mL of toluene was added to each mixture for extraction. Upper part of the mixture was extracted with toluene and the absorbance was measured in the spectrophotometer (model: 2100UV, UNICO, USA) at 520 nm.

Na+ and K+ content were determined using Borgan's (2006) method. Initially, 1 g of dried leaf tissue was dry-ashed in an electric furnace at 500°C for 4 h. Then, 10 mL of 1 N hydrochloric acid was added to each sample and heated to boiling point until it was completely evaporated. Then 10 mL of distilled water was added to each one of the samples, and they were placed on the heater, then removed as soon as they start boiling. Finally, their volume was made up to 50 mL by using distilled water, and flame spectrometer was used to measure concentration of elements.

Antioxidant enzyme assays

Antioxidant enzymes were extracted from frozen leaf tissues as described in Sabra *et al*. (2012). Frozen leaf tissue (0.2 g) was crushed into a fine powder using pestle and mortar and then homogenized with 5 mL of 100 mM K-phosphate extraction buffer (pH = 7.0) containing 1 mM EDTA.Na2 and 1% poly-vinyl-pyrrolidone (PVP). The homogenates were centrifuged at 14000 rpm for 20 min at 4°C. The supernatant was collected and stored at "20 °C until analysis.

The CAT activity was determinated as described by Sabra *et al*. (2012) with monitoring the disappearance of H2O2. The reaction was initiated by adding 50 µl of enzyme extract to 1.5 mL of reaction mixture containing 50 mM K-phosphate buffer (pH= 7.0), and 15 mM freshly prepared H2O2. The decrease in absorbance was measured at 240 nm for 1 min, and the degradation of 1 µmol H2O2 per min was defined as one unit of CAT. The POX activity was measured based on the method of the Aganchich *et al*. (2007). For this measuremet, 100 µL sample was added to 1 mL of 20 mM sodium phosphate buffer (pH= 7) which contained 276 µL of guaiacol per 50 mL buffer. The reaction was initiated by adding 200 µL of 0.03% H2O2 to distilled water (w/w). The reaction was mixed and the absorbency of the solutions at 470 nm was measured after 2 min using a spectrophotometer. The PPO activity was measured according to Aganchich *et al*. (2007). The reaction mixture was contained 200 µL of enzyme extract, 2 mL of catechol 1% and 1.8 mL of 0.05 M sodium phosphate buffer (pH= 7). The reaction mixture was absorbed and was measured after 2 min at 410 nm.

Total protein measurement

Protein was estimated according to Bradford *et al*. (1976) method. This method used 0.2 g of frozen leaf tissues and mixed with 1.2 ml Tris-HCl buffer (pH=7.5), homogenized and centrifuged at 14,000 rpm for 20 min at 4 °C. Bradford reagent was added to the supernatant and the absorbance was measured at 595 nm. For the last two measurements, standard solution were constructed by using BSA (bovine serum albumin, concentration of 0.1 - 1%) and the absorbance was measured on the spectrophotometer.

Essential oil

Essential oil content was determined through hydrodistillation by submitting 100 g of aerial part of dried plants to a modified Clevenger apparatus (Ozturk *et al*. 2004). After 3 hours of distillation, essential oil ratio was measured by using dry yield (biomass yield) of peppermint.

Statistical analysis

The mean values for all parameters were obtained from three replications, and the standard error of the means was calculated. Two-way ANOVA and Duncan's test were used to compare the significance of the mean values of treatments. Data were analyzed with SPSS 16 (SPSS Inc., Chicago, IL, USA) and Sigma Plot v. 11 was used for calculating the type of regression equation. R2, R2adj and RMSE were applied to determine the best estimates of the parameters. R2 was determined by following formula:

R2= SSR/SST

Where SSR denotes the sum of squares (SS) for regression ($\sum_{i=1}^{n} L - \bar{L}$) and SST denotes the total SS ($\sum_{i=1}^{n} Li - \bar{L}$). Li is the observed value and 8 is the corresponding estimated value:

R2adj = R2 - $\frac{k-1}{n-k}$ (1-R2)

Where N is number of observation, k number of parameter.

RMSE = $\sqrt{(\frac{1}{n})\sum(Yobs - Ypred)_2}$

Where Yobs denotes observed value, Ypred is predicted value, and n is the number of samples. Following equations were used for:

1- f =y0+a*exp (-b*x)

a = max parameter, b = slope and yo= constants.

2- f = a*exp(-.5*((x-x0)/b)^2)

a = max parameter, b = slope and x0= salinity dose that provides max parameter.

3- f = y0+a*exp(-.5*((x-x0)/b)^2)

a = max parameter, b = slope and x0= salinity dose that provides max parameter and yo= constants (Archontoulis and Miguez, 2013).

Table 1. Select chemical characteristics of the Hoagland's solution used in the experiment

Component	Stock Solution (mg/L)
	Macro Nutrient
Ca(NO3)2.4H2o	738
KNO3	464.6
MgSO4	180.75
MgSO4.7H2o	369.75
KH2Po4	198.4
NH4.NO3	34.28
	Micro Nutrient
MnCl2.4H2o	1.8
ZnSO4.7H2o	2.2
H3BO2	2
Na2MoO4.2H2o	0.01
Iron	4
CuSO4.5H2o	1.2

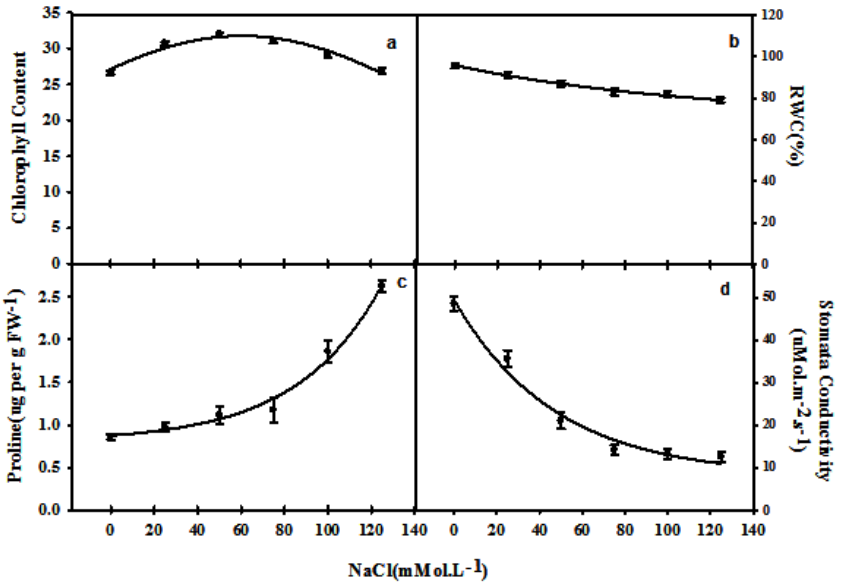

Fig. 1. Effects of salinity on total chlorophyll content (a), RWC (b), proline content (c) and stomata conductivity (d), of peppermint leaves. Data are means of three replications and vertical bars indicate Standard Error. Mean values with different letters are significantly different by the Duncan test (Pd"0.01)

RESULTS

Chlorophyll content and Stomata conductivity

Experiment results showed that salinity had a significant effect on chlorophyll content and stomata conductivity of peppermint leaves (Fig. 1). Changes in chlorophyll content under salinity stress were peak function. Moderate stress increased chlorophyll content, thus highest chlorophyll content (31.77) was recorded by NaCl 60 mM (Table 2). Salinity reduced stomata conductivity of peppermint leaves. Single equation regression was the best fit for stomata conductivity changes under salinity condition. The stomata conductivity was 41.29 µmol.m-2.s-1 under non-stress condition (NaCl= 0), and decreased by a slope of 0.021 and reached 8.11 µmol.m-2.s-1 under full stress (Table 2).

Osmotic adjustment

RWC and proline content of peppermint leaves were affected significantly by salinity stress (Fig. 1). Plants grown under salinity condition had higher proline and lower RWC than plants grown under non-stressful condition. RWC change was single under stress condition, thus salinity reduced RWC by a slope of 0.008 and loss RWC to 79.08% under salinity 125 mM (Table 2).

Table 2. Estimates parameter function for total chlorophyll, stomata conductivity, RWC and proline content of peppermint under salinity stress condition

Estimates parameter						Function equation	Characteristics
RMSE	Radj	R2	X0 or y0	b	a		
0.64	0.91	0.94	60.5± 2.6	107.43± 7.3	31.77± 0.41	f=a*exp(-.5*((x-x0)/b)^2)	Chlorophyll
2.61	0.96	0.98	8.06 ± 3.9	-0.02± 0.005	41.37± 4.14	f=y0+a*exp(-b*x)	Stomatal
0.60	0.99	0.99	70.62± 4.3	-0.008± 0.002	95.19± 4.15	f=y0+a*exp(-b*x)	RWC
0.10	0.97	0.98	0.80± 0.11	0.02± 0.005	2.65± 2.04	f=y0+a*exp(b*x)	Proline

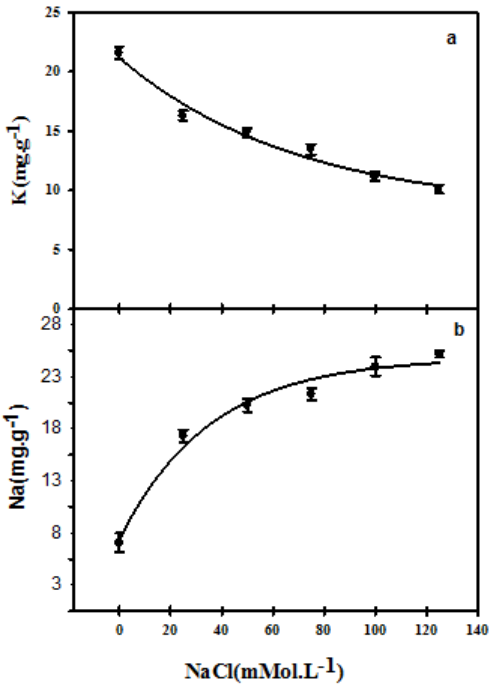

Fig. 2. Effects of Salinity on K+ (a) and Na+ (b) content of peppermint. Data are means of three replications. Mean values with different letters are significantly different at P = 0.01 (the Duncan test)

Salinity stress caused increasing in proline content with a single equation (Fig.1). Highest proline content (2.62 µg per g FW-1) was observed in salinity level of 125 mM which demonstrated approximately three fold over control (Table 2).

As expected, salinity significantly affected Na+ and K+ contents of peppermint leaves. Changes of these element contents were consistent with the single equation (Fig. 2). Salinity increased Na+ content from 6.54 mg per g DW-1 under non-stress condition by a slope of 0.028 to 24.68 mg per g DW-1 under stressful condition (125 mM) (Table 3). Also, salinity reduced K+ content by a slope of 0.014 and from 21.56 mg per g DW-1 to 10.06 mg per g DW-1 (Table 3).

Antioxidant enzyme activity

Moderate stress increased the activities of CAT and POX in peppermint; while the trend was decreasing for higher levels of salinity stress. Based on the estimations, the highest activity of CAT and POX enzymes were observed at 54.28 mM and 53.19 mM of salinity, respectively, and

the least activity was observed in control treatment (Fig. 3). For both enzymes, low concentration of salinity resulted in increased enzyme activity and by increasing the salinity concentration, a reduction in the activity of enzymes was observed. The activity of PPO revealed an increasing trend with increasing of NaCl concentration. The single equation was the best-fit equation for this enzyme activity changes under stress conditions. The lowest activity detected in control condition. Salinity increased the activity of PPO by a slope of 0.017 and reached the highest content (65.39 µmol per mg protein -1 min-1) in the salinity level of 125 mM (Table 4).

Protein content

Total protein content was significantly affected by salinity stress (Fig. 3). The single equation was a suitable equation for the process of changes in total protein content. The highest protein content was observed in non-stress condition (4.2 mg per g FW-1). That decreased and dropped to 1.23 mg per g FW-1 under stressful condition (125 mM) (Table 4).

Dry weight and Essential oil

The results showed that growing peppermint plants under salinity condition had a significant effect on dry weight; essential oil percentage and oil yield (Fig. 4). Salinity resulted in decreased dry weight of peppermint and decreasing had a single equation. Dry weight was 11.34 g under non-stress condition; salinity caused a decreasing by a slope of 0,014and the dry weight reached to 4.24 g under stressful condition (125mM) (Table 5).

During the experimental period, moderate salinity stress increased essential oil percentage;

Table 3. Estimates parameter function for Na+ and K+ elements content of peppermint under salinity stress condition

Estimates parameter						Function equation	Characteristics
RMSE	Radj	R2	X0 or y0	b	a		
1.18	0.96	0.98	6.84± 1.16	0.028± 0.006	17.46± 1.52	f=y0+a*(1-exp(-b*x))	Na+
0.8	0.96	0.97	8.22± 2.32	0.014± 0.005	12.96± 2.17	f=y0+a*exp(-b*x)	K+

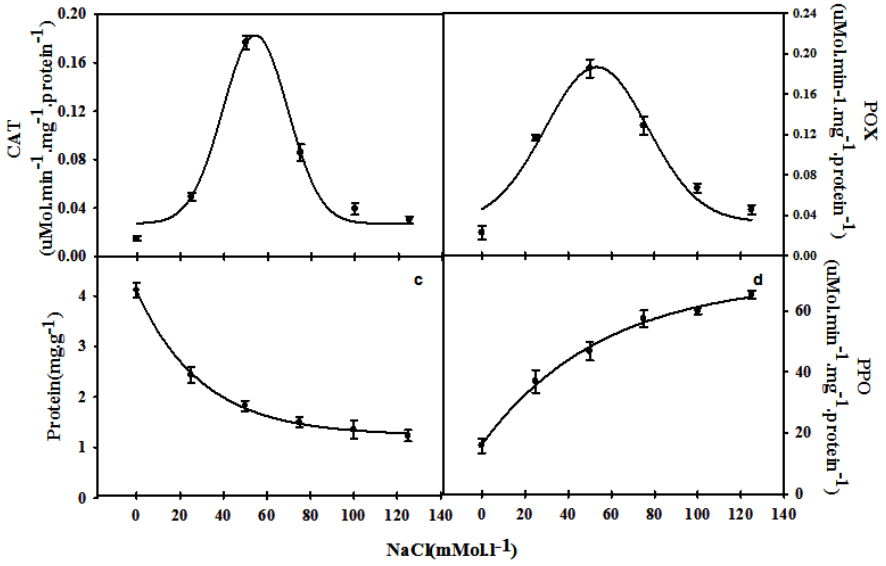

Fig. 3. Effects of salinity on CAT (a), POX (b), total protein content (c) and PPO (d), enzymes of peppermint. Data are means of three replications and vertical bars indicate Standard Error. Mean values with different letters are significantly different at P = 0.01 (the Duncan test)

however, changes in the essential oil percentage in peppermint leaves followed peak trend (Fig. 4). Maximum essential oil was 1%, which was related to 53.36 mM salinity. The minimum essential oil 0.85% was related to stressful condition (Table 5). We found a significant decrease in essential oil yield in peppermint due to salinity stress. Change in oil yield was linear, thus resulted in highest (9.78 g plant-1) and lowest (4.6 g plant-1) oil yield observation in non-stress and full stress condition, respectively (Fig. 4).

DISCUSSION

Previous research has showed that salinity affects different aspects of plant metabolism,

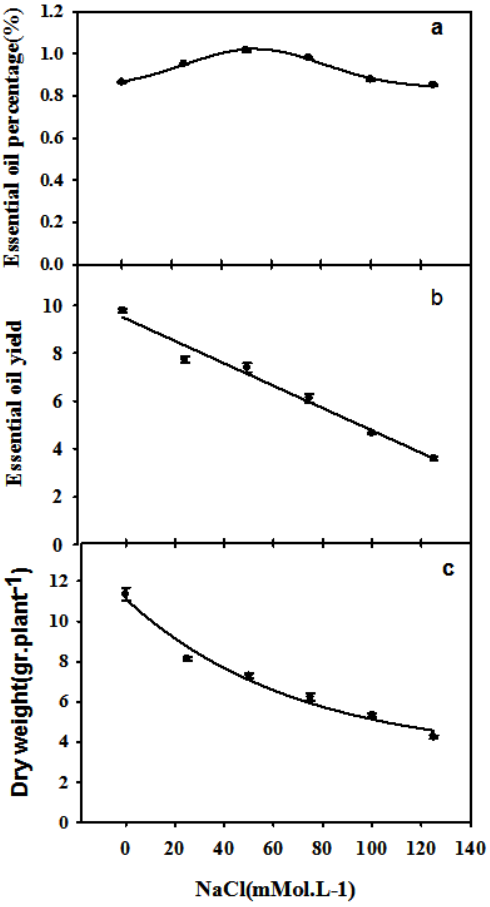

Fig. 4. Effects of salinity on dry weight (a), essential oil percentage (b) and essential oil yield (c) of peppermint. Data are means of three replications. Mean values with different letters are significantly different at P = 0.01 (the Duncan test)

Table 4. Estimates parameter function for total protein content, PPO, CAT and POX enzymes activity of peppermint under salinity stress condition

RMSE	Radj	R2	Estimates parameter X0 or y0	b	a	Function equation	Characteristics
0.05	0.99	0.99	1.23± 0.04	0.031± 0.002	2.86± 0.06	f=y0+a*exp(-b*x)	Protein
1.57	0.99	0.99	16.15± 1.52	0.017± 0.002	54.24± 3.00	f=y0+a*(1-exp(-b*x))	PPO
0.012	0.95	0.98	54.28± 2.01	14.89± 1.86	0.15± 0.014	f=y0+a*exp(-.5*((x-x0)/b)^2)	CAT
0.021	0.88	0.95	53.19± 3.54	23.71± 5.36	0.15± 0.021	f=y0+a*exp(-.5*((x-x0)/b)^2)	POX

Table 5. Estimates parameter function for dry weight, essential oil percentage and essential oil yield of peppermint under salinity stress condition

RMSE	Radj	Estimates parameter R2	y0	b	a	Function equation	Characteristics
0.48	0.96	0.97	3.28± 1.37	0.014± 0.005	7.83± 1.28	f=y0+a*exp(-b*x)	Dry weight
0.009	0.98	0.99	53.36± 1.4	27.58± 2.64	0.18± 0.01	f=y0+a*exp(-.5*((x-x0)/b)^2)	Essential oil
0.36	0.97	0.97	9.50 0.26±	-0.04± 0.003	-	f = y0+a*x	Oil yield

inhibiting nitrogen uptake (Hawkins and Lewis, 1993), water uptake (Koyro, 2006), ion distribution (Rogers and Noble, 1997), mineral assimilation (Keutgen and Pawelzik, 2009), secondary metabolite production (Chen *et al*., 2014), stomatal conductance and photosynthesis (Turan *et al*., 2009 and 2010), and plant growth and yield (Muhling and Lauchli, 2002). We showed in this study that the physiology of peppermint plant was affected slightly by moderate levels of salinity; however, server salinity stress influenced dramatically the different physiological processes of treated plants.

We found that peppermint plants grown under stressful condition produced poor dry weight compared to non-stress and moderate stress condition (Fig. 4). According to the reports, the reduction in dry weight under saline stress may be attributable to the inability of the plant to absorb water and nutrients and to suppression of growth during the early developmental stages (Roodbari *et al*., 2013; Eziz *et al*., 2013). Also, salinity induces osmotic stress, specific ion toxicity, and ionic imbalances, reducing the plant growth or causing plants to die ([a]oban and Baydar, 2016).

Salt stress is believed to decrease chlorophyll content (Turan *et al*., 2009); however, in our experiments, chlorophyll content was initially increased in 50mM NaCl concentration and then reduced. The higher chlorophyll content is associated with the higher tolerance for salt stress (Shirazi *et al*., 2009). Chlorophyll biosynthesis is the crucial factor to achieve oxygenic photosynthesis (Tsukatani and Masuda, 2015). Fariduddin *et al*. (2013) argued that reduction of chlorophyll content under salinity stress might be due to the increased activity of chlorophyll-degrading enzyme (chlorophyllase), the decreased uptake of Mg element, or the stomatal closure. Also, salinity resulted in leaf yellowing, browning, and falling, which was correlated with NaCl concentrations. Most plants treated with 125 mM NaCl died. High-NaCl–induced plant death has been reported for peppermint ([a]oban and baydar, 2016).

Contents of different elements, as another physiological response, were changed in salinity stress. In addition, K+ and Na+ contents provide useful measures for assessing salinity-associated damage. High concentrations of salt in the external solution disturb ionic balance and ion homeostasis

(Parida and Das, 2005). There is a competition between Na+ and K+, which leads to a reduction in the level of internal K+ at high concentrations of external NaCl and results in increased Na+ increase in plant tissue (Rus *et al*., 2004; Kaya *et al*., 2007). Potassium uptake is influenced by NaCl and leads to increased Na+/K+ ratio, which in turn inhibits plant growth and causes ionic toxicity (Cuin *et al*., 2003). In our study, increased NaCl concentration significantly decreased potassium uptake and increased Na+/K+ ratio. Sodium-associated impairment of potassium uptake of K+ is believed to be the result of chemical similarities between these ions (Borsani *et al*., 2003). Proline serves as an energy supply, has antioxidant properties, and exerts osmotic effects as well (Chookhampaeng, 2011). It is postulated that proline may contribute to stabilization of DNA, protein, and membrane structure and function (Kavi Kishor *et al*., 2005). This might be the reason for the observed increases in proline content in plants in response to stress factors. We found that proline content in plants grown under stress condition were dramatically higher compared with plants grown under control condition (Fig. 1). Therefore, it can be concluded that higher proline accumulation may be associated with increased stress tolerance. Our finding is consistent with the previous observations that high salinity results in increased proline content (Gorai *et al*., 2010; El-Danasoury *et al*., 2010).

Plants have multiple mechanisms for stress tolerance. Change in soluble protein content is one important response to salt stress (Ghorbanli *et al*., 2012). The soluble protein content of peppermint decreased with increasing the level of salinity stress, suggesting a dose-dependent inhibition of protein synthesis (Fig. 3). This actually may be quite reasonable because plants may have to limit energy expenditure under salinity stress (Huang *et al*., 2013).

Plants use enzymatic and non-enzymatic antioxidant to response to salinity stress ([a]oban and Baydar, 2016). Based on our results, moderate stress increased the activities of CAT and POX in peppermint; however, severe stress decreased those activities. Also, the activity of PPO revealed an increasing trend with increasing of the NaCl cocentration. Ibrahim *et al*. (2015) reported that antioxidant enzymes such as CAT, POX, and PPO can scavenge ROS under salt tolerance. Anincrease in antioxidant enzymes of wheat under stress condition was reported by Gong *et al*. (2005). El-Danasoury *et al*. (2010) reported similar results in their experiments on M. piperita. Superoxide radicals, the toxic products of oxidative metabolism, may interact with H2O2 to form highly reactive hydroxyl radicals that are thought to be primarily responsible for oxygen toxicity in the cell (Uchida *et al*., 2002). Other studies reported that the process of transformation of H2O2 into molecular oxygen and water is catalyzed by the CAT enzyme found in peroxisome and cytosol (Gill and Tuteja, 2010); and this CAT enzyme protects cells from oxidative effects of H2O2 and OH radicals.

Essential oils comprise a wide range of secondary metabolites. In our study, salt stress caused an initial increase in essential oil content at low salinity, but then significantly decreased essential oil content (Table 5). This seems to be due to peppermint tolerance in low salinity and essential oil role as a defensive compound. Then, with increasing NaCl concentration, the peppermint lose tolerance and essential oil biosynthesis process is plagued. Tabatabaie *et al*. (2007), Aziz *et al*. (2008), and Khorasaninejad *et al*. (2010) also reported significant decreases in total essential oil content in peppermint plants stressed with NaCl compared with the controls. These results support the notion that higher NaCl concentrations suppress essential oil biosynthesis in peppermint. As suggested by Aziz *et al*. (2008), reduced essential oil content could be a consequence of a reduction in photosynthesis rate or additional changes in metabolic systems. Any disruption in metabolic functions could impair oil biosynthesis, resulting in reduced essential oil content (Srivastava *et al*., 1998). We hypothesized that peppermint was semi-tolerant to salinity stress. The results obtained in this study indicated that plants grown under lower NaCl concentration had the similar dry weight and essential oil yield to the plant grown under non-stress condition.

CONCLUSION

In summary, results obtained in this study indicated that peppermint is not a salinity tolerant

medicinal plant. However, in moderate salinity, it is possible to grow it for harvesting dry weight and essential oil yield.

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