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The Effect of Salinity Stress on Growth Parameters and Essential oil percentage of Peppermint (*Mentha piperita* L.)

Nasim Roodbari [*1], Shahram Roodbari [2], Amin Ganjali [3], Fariba Sabeghi nejad [4] and Masoumeh Ansarifar [5]

[1] Department of Experimental science, Kahnouj Branch, Islamic Azad University, Kerman, Iran, [2]Master of Agricultural Management Agriculture of Jiroft, Iran, [3]Faculty of Experimental science, Kahnouj Branch, Islamic Azad University, Kerman,Iran, [4]Master of Agriculture of Experimental science, Kahnouj Branch, Islamic Azad University, [5] Faculty of computer, Kahnouj Branch, Islamic Azad University, kerman, Iran

ABSTRACT

Peppermint (*Mentha piperita* L.) is one of the most important plants producing essential oil. An experiment was carried out using a randomized complete block design with 3 replications to study the effect of salinity stress on growth parameters, essential oil percent of peppermint. 4 levels of salinity solution containing 0, 50, 100 and 200 mmol NaCl was applied in irrigation plantings in a greenhouse. The results showed that salinity stress significantly affected Stem length, root length, shoot wet weight, root wet weight and shoot dry weight, root dry weight, Internodes length, biomass and Essential oil percent in $P < 0.05$. An increase in the salinity lead to reduce in length of stem and root, fresh weight of stem and root, dry weight of stem and root, internodes length, total biomass and essential oil percent. It is necessary to mention that peppermint did not tolerate 200 mmol NaCl and died under this condition. The highest values of growth parameters and essential 1 oil percent were observed under non-salinity condition(control). Also, were increased proline with the increase of salinity($P < 0.05$).

Key words: Essential oil, Peppermint, Sodium chloride, Growth parameters, Proline.

INTRODUCTION

Recently, medicinal and aromatic plants have received much attention in several fields such a agroalimentary, perfumes, pharmaceutical industries and natural cosmetic products (Olfa et al,2009). Although, secondary metabolites in the medicinal and aromatic plants were fundamentally produced by genetic processing but, their biosynthesis is strongly influenced by environmental factors (Yazdani et al.2002). It means biotic and abiotic environmental factors affect growth parameter, essential oil yield and constituents(Aziz et al.2008b and Clark et al.2008). Abiotic environmental stresses especially salinity and drought has the most effect on medicinal plants (Heidari et al.2008). The different results were dedicated from the effect of salinity stress on the quantitative and qualitative parameters. For instance, it was found that increasing of salinity stress decreased almost all of growth parameters in *Nigella sativa*, some growth parameters and essential oil amount in Chamomile (Razmjoo et al.2008)

Corresponding Author E-mail: Roodbari.nasim7@gmail.com

and essential oil yield in Lemon Balm (Ozturk et al.2004). Also, effect of salinity parameter on essential oil quality in Lemon Verbena showed the increased amount of geranial as salinity level was increased (Tabatabaie and Nazari.2007). In the other hand, findings of the previous researchers about stress are contradictory. Peppermint (Mentha piperita L.) belongs to mint (Lamiaceae) family and is herbaceous and perennial considered as a medical and aromatic plant and were produced extensively for the medicinal and food product industries (Yazdani et al.2002 and Grieve. 1999). Fundamental components of peppermint essential oil include menthol, menthone, methylacetat, menthofuran and pulegone (Tabatabaie and Nazari. 2007and Mahmoud, and Croteau.2003). It is necessary to mention that the amount of peppermint essential oil and its constituents considerably were impressed by different factors such as climate, soil type, geographical area(Yazdani et al.2002) harvest time and fertilizer usage (Niakan et al.2004). In this study, the effect of salinity stress is investigated on growth parameters, essential oil constituents and proline in peppermint. The results of this study can be used by producers of this plant to be able to access desirable quantitative and qualitative properties in its essential oil in order to its optimum applications in industry.

MATERIALS AND METHODS

In this study the effect of salinity stress on growth and production characteristics of Peppermint essential oil was done experiment under greenhouse conditions. peppermint plants were initiated from rhizome cuttings(10 cm long) supplied by Azad University of Jiroft Branch. They were transferred into laboratory of biology and them were sterilized of laminar air flow system in the following order with 10% ethanol, 15% sodium hypochlorite for 2-3 minutes each and washed 3 times with sterile water and were grown in pots with dimensions(25 cm head diameter × 20 cm height) and all of them were filled with sandy loam soil. This experiment was carried out using a randomized complete block design with 3 replications. The factors studied included 4 salinity levels that were used the certain amounts of sodium chloride in distilled water containing levels 0(S_1 or control), 50(S_2), 100(S_3) and 200(S_4) mM sodium chloride in distilled water. The total electric conductivity (E.C.) of the culture media at 25°C was 0/71, 6/72, 12/91 and 15/36 dS/m, respectively. Each experiment was repeated in triplicate. Published research supports the premise that the salinity of water plants will actually have available to utilize, the soil EC, ECe, or ECsat(saturated paste extract), is on average as much as three times the salinity of applied irrigation water due to evapotranspiration(Ayers and Westcot,1976). Initially, all pots watered with distilled water until 7-8 leaf stage of the seedlings. Then, the number 3 seedlings were maintained had better growth than the rest of the into pots. The irrigation with saline solution was applied according need pots in a 3 months period. At the end of the experiment, selected of the 4 plants in each treatment that were the same for both sizes and were measured the parameters such as shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, Internodal length , essential oil content and proline content. Essential oil content was determined by hydro distillation method by submitting aerial part of dried plants (100g) in modified Clevenger apparatus (Ozturk et al.2004). After 3 hours distillation was stopped so essential oil ratio was measured by using dry yield (biomass yield) of peppermint. Before the oil, were measured moisture samples, placing it in the oven (105 ·C) for 3 h. Also, For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100°C for 1 hour. The reaction was arrested in an iced bath and the cromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined in a BioMate spectrophotometer (Thermo Spectronic). The concentration of proline in root tissues was determined by triturating the frozen roots. Subsequently, 0.5 g of triturated roots was mixed with 2 ml of 3% sulfosalicylic acid. The supernatant after centrifugation was mixed in a 1:1:1 ratio with ninhydrin acid and glacial acetic acid. The reaction and determination of proline were carried out similarly to that described above. The method was calibrated for each determination with standard proline solutions within the detection range of the method (0-39 µg. ml). Data were subjected to analysis of variance using statistical analysis system and followed by Duncan's multiple range tests and terms were considered significant at $P < 0.05$ by SAS software.

RESULTS AND DISCUSSION

The results showed that salinity stress significantly affected growth parameter, essential oil percent and proline content of peppermint in $P < 0.05$ (Table 1). Highest Shoot length, root length, shoot wet weight, root wet weight, shoot dry weight, root dry weight, internodes length, Essential oil percent were achieved under S_1 treatment and with increasing in salinity from 0 to 200 mmol L^{-1} NaCl, all of growth parameter, essential oil percent were reduced (Figures1). Peppermint did not tolerate 200 mmol L^{-1}NaCl and died under these conditions. As it was shown in the results of this study, salinity stress had a negative effect on most of the morphological characteristics under study, as with increase in salinity from 0 to 200 mmolL^{-1} NaCl, plant stem and root length, internodes length were reduced. Salinity also caused reduction in the shoot and root fresh weight, shoots and root dry weight and essential oil. In this study, was not essential oils in the highest level of stress, due to a very low tissue fresh mint(Table 1). The main reason for this reduction may be attributed to suppression of growth under salinity stress during the early developmental stages (shooting stage) of the plants. Salinity stress significantly decreased Essential oil percentage. Olfa et al. (2009) also showed that oil content in Marjoram (Origanum majorana) was decreased consistently with increase in external salt levels. Reduced stem length, shoot wet and dry weight and biomass may have resulted in oil content reduction of peppermint under salinity stress environment. Salinity tolerance in plants is based on the Sensitive Plants(injury at>2 dS/m ECe) and Moderately tolerant (injury at 4-6dS/m ECe)(Wolf, 1999). The highest and lowest of proline content was observed in the 200 and 0 mmol $L^{\cdot}$ NaCl treatments(Figures2).The increase in proline content could be attributed to a decrease in proline oxidase activity in saline conditions (Muthukumarasamy et al,2000). Proline occurs widely in higher plants and accumulates in larger amounts than other amino acids. Plants under salt stress may present increased levels of certain compounds. Amino acids (alanine, arginine, glycine, serine, leucine, and valine, together with. This result is consistent with the amino acid, proline, and the non-protein amino acids, citrulline and ornithine) and amides (such as glutamine and asparagines) have also been reported to accumulate in plants subjected to salt stress (Mansour, 2000). Those reported by Aziz et al. (2008a) for decreasing in growth parameter and essential oil of peppermint grown in different levels of salinity stress. As stated by Munns(2003), suppression of plant growth under saline conditions may either be due to decreased availability of water or to the toxicity of sodium chloride. Also the reduction in dry weight under salinity stress may be attributed to inhibition of hydrolysis of reserved foods and their translocation to the growing shoots. Salinity stress imposes additional energy requirements on plant cells and less carbon is available for growth and flower primordial initiation and then less essential oil may be synthesized (Cheesman.1988). Safikhani et al.(2007) showed that increasing salinity stresses caused a reduction, both in shoot and root yield of Nigella sativa. Khammari et al (2007) also compared the performance of six medicinal plants, Cyamopsis psoraloides, Cynara scolymus, Hibiscus sabdariffa, Cassia angustifolia, Ocimum basilicum and Hyssopus officinalis at germination stage at different levels of NaCl salinity. They concluded that salinity resulted in the suppression of plant growth in all species. The ability to limit Na transport into the shoots and to reduce the Na accumulation in the rapidly growing shoot tissues, is critically important for maintenance of high growth rates and protection of the metabolic process in elongating cells from the toxic effects of Na+(Razmjoo et al.2008). However, this characteristic was not considered in this study. From a qualitative point of view, menthol and menthone are the main constituents of the peppermint essential oil. The commercial importance of peppermint essential oil depends on the percentage of these 2 components as well as the low percentages of other undesirable compounds such as menthofuran (Mahmoud and Croteau.2003). An excess of soluble salts in the soil leads to osmotic stress, specific ion toxicity and ionic imbalances (Munns.2003) and, as a consequence, plant can go to death (Rout and Shaw,2001). All seedlings of melissa officinalis died at 6 dS/m (Ozturk et al,2004); the observed increasing soil salinity up to 3000 ppm resulted in complete death of sage plants (Hendawy and Khalid,2005). Salinity reduces the production of essential oils in the mint(Lamiaceae)family plants and This is probably due to the limited supply of cytokinin from the roots to the branches and the leaves are changing ratio between Abscisic acid and cytokinin(Charles.1990).

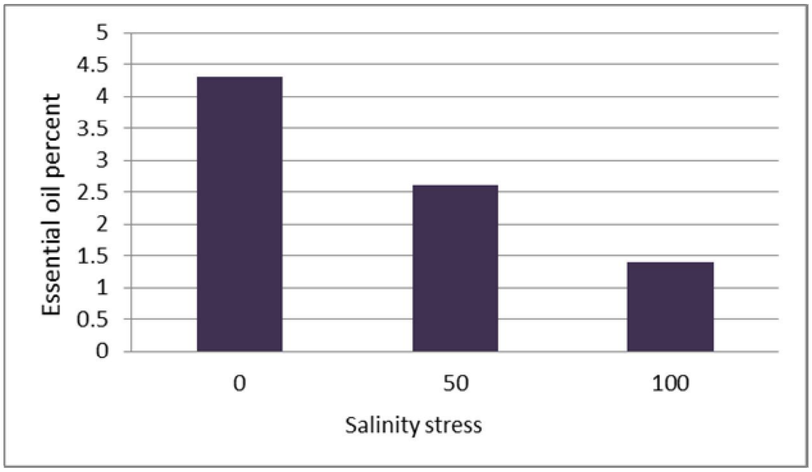

Fig 1: Essential oil percentage under different salinity stress

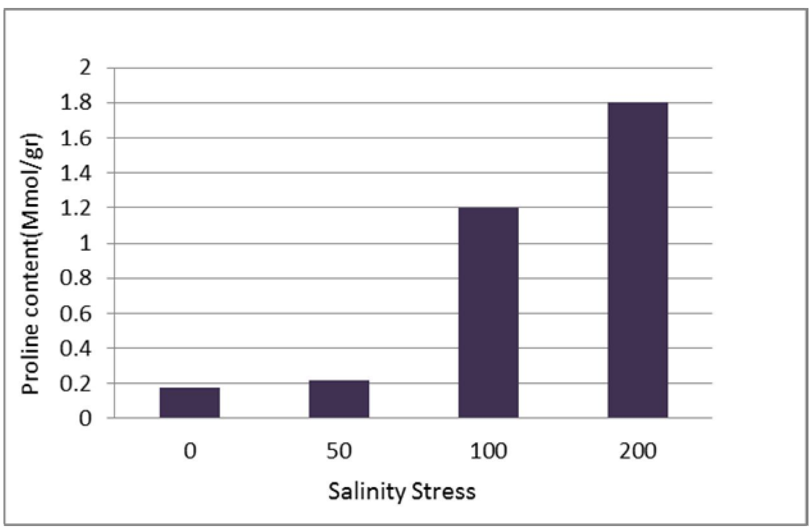

Fig 2: Proline content under different levels of salinity stress

Funding: Peppermint was moderately tolerant to salinity, because salinity inhibited various growth parameters of this plant to various degrees. Peppermint Peppermint can be grown successfully on most agricultural soils, as long as NaCl does not exceed the critical values (200 mmol in this study).

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Table 1: Effect of salinity levels on growth parameters of Peppermint

				Mean-square					
Salinity (mm)	Stem Length (cm)	Root length(cm)	Shoot wet Weight(gr)	Shoot dry Weight (gr)	Root wet Weight (gr)	Root dry Weight (gr)	Internodes length(cm)	Essential oil percent	Proline content (Mmol/gr)
0(s_1)	13.7 [a]	14.4[a]	5.71[a]	3.96[a]	6.8[a]	1.33[a]	5.6[a]	4.3[a]	0.18[d]
50(S_2)	9.8[b]	8.2 [b]	5.33[a]	3.24[a]	3/08[b]	1.7[a]	4.8[ab]	2.6[b]	0.22[c]
100(S_3)	6.7[c]	5.2[c]	5.56[a]	2.63[b]	2.01[c]	1.06[a]	3.7[b]	1.4[c]	1.2[b]
200(S_4)	4.2 [d]	3.1[d]	5.26[a]	2.11[b]	1.1[d]	1.01[a]	1.5[c]	--	1.8[a]
Significance	*	*	ns	*	*	ns	*	*	*

[a,b,c,d] Different superscripts in a raw differ significantly. ns= no significant,* significant(P<0.05).