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

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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 constituents and yield of peppermint. Full-strength Hoaglund's solution containing 0, 50, 100 and 150 mmol L^{-1} NaCl was applied in irrigation plantings for 4 months 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, stolon length, biomass, Essential oil yield 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 and yield. It is necessary to mention that peppermint did not tolerate 150 mmol L^{-1} NaCl and died under this condition. The highest values of growth parameters and essential oil percent and yield were observed under non-salinity condition (control). Also, the highest values of Mentone were obtained under non-salinity condition (control) by using GC-MS.

Key words: Peppermint · Mentone · Essential oil · Hoaglund's solution · GC-MS · Salinity

INTRODUCTION

Recently, medicinal and aromatic plants have received much attention in several fields such as agroalimentary, perfumes, pharmaceutical industries and natural cosmetic products [1]. Although, secondary metabolites in the medicinal and aromatic plants were fundamentally produced by genetic processing but, their biosynthesis is strongly influenced by environmental factors [2]. It means biotic and abiotic environmental factors affect growth parameter, essential oil yield and constituents [3, 4]. Abiotic environmental stresses especially salinity and drought has the most effect on medicinal plants [5]. 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 [6] and essential oil yield in Lemon Balm [7]. Also, effect of salinity parameter on essential oil quality in Lemon Verbena showed the increased amount of geranial as salinity level was increased [8]. 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 [2, 9]. Fundamental components of peppermint essential oil include menthol, menthone, methylacetat, menthofuran and pulegone [8, 10]. It is necessary to mention that the amount of peppermint

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essential oil and its constituents considerably were impressed by different factors such as climate, soil type, geographical area [2] harvest time and fertilizer usage [5,8,11]. In this study, the effect of salinity stress is investigated on growth parameters, essential oil constituents and yield 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

The peppermint plants were initiated from rhizome cuttings (10 cm long) supplied by Jahad daneshgahi in March 2009. They were transferred into pots filled with cocopite and perlite (2:1 v). Initially, all plants were fed with a nutrient solution with half strength Hoagland's solution [12] and then after a week, Hoagland's solution with four different salinity levels 0(S_1), 50(S_2), 100(S_3) and 150(S_4) mmol L^{-1}NaCl was prepared. This experiment was carried out using a randomized complete block design with 3 replications. Irrigation with 0.5 liter of saline solution was started at shooting stage in each pot and then irrigation with saline solution was applied every day in a four months period. At the end of the experiment (in july2009), all the plants within each plot were harvested for the study of their Stem length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, Internodal length, stolon length, shoot to root ratio, biomass, essential oil yield and essential oil percent. Essential oil content was determined by hydro distillation method by submitting aerial part of dried plants (100g) in modified Clevenger apparatus [7]. After 3 hours distillation was stopped so essential oil ratio was measured by using dry yield (biomass yield) of peppermint. The composition of essential oil was analyzed by GC-MS using an Agilent 6890 gas chromatograph mass spectrometer. The operating conditions were as follows: carrier gas, helium with a flow rate of 0.8 ml/min; column temperature, 5 min in 50 °C, 240 °C at 15 °C/min and finally 3 min in 300 °C, injector temperature, 290 °C; detector temperature, 220 °C. The identification of the GC peaks corresponding to the components of the essential oil was based on direct comparison of the retention times (RT) and mass spectral data with those for standard compounds. Data were subjected to analysis of variance (ANOVA) using statistical analysis system and followed by Duncan's multiple range tests and terms were considered significant at $P < 0.05$ by SPSS software.

RESULTS AND DISCUSSION

The results showed that salinity stress significantly affected growth parameter, essential oil yield and constituents of peppermint in $P < 0.05$ (Table 1). Highest Stem length, root length, shoot wet weight, root wet weight, shoot dry weight, root dry weight, Internodes length, stolon length, biomass, Essential oil yield and Essential oil percent were achieved under S_1 and With increasing in salinity from 0 to 100 mmol L^{-1} NaCl, all of growth parameter (except of shoot to root ratio), essential oil yield were reduced (Figures 1, 2). Peppermint did not tolerate 150 mmol L^{-1} NaCl and died under these conditions. In peppermint, the major constituents of the essential oil were menthone and menthol, which together accounted for approximately 58%-66.2% of total oil composition in each treatment (Table 2). The highest and lowest proportion of menthone was observed in 0 and 50 mmol L^{-1} NaCl treatments, respectively. Significantly higher levels of menthone were observed in the 0 mmol L^{-1} NaCl. However, the proportion of menthol was reduced in that treatment. 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 100 mmolL^{-1} NaCl, plant stem and root length, internodes length and stolon length were reduced. Salinity also caused reduction in the shoot and root fresh weight, shoots and root dry weight and biomass. The main reason for this reduction may be attributed to suppression of growth under salinity stress during the early developmental stages (shooting stage) of

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

Salinity (mm)	stem length (cm)	root length (cm)	shoot fresh weight (gr)	root fresh weight (gr)	shoot dry weight (gr)	root dry weight (gr)	Internodes length (cm)	stolon length (cm)	Shoot/ root	biomass	Essential oil yield (ml/plant)	Essential oil percent
0(S1)	80a	31.66a	63a	29.66a	11.86a	9.23a	4.33a	51.38a	3.1a	21.09a	0.78a	0.1623a
50(S2)	40b	22ab	6.56b	3.63b	1.63b	1.2b	2b	27.66ab	2.29a	2.72b	0.42c	0.0113b
100(S3)	42b	14.66b	8b	3.2b	0.8b	3.7b	1.33b	3.46 b	2.04a	1.11b	0.53b	0.0057b
Significance	**	**	**	**	**	*	**	*	ns	**	**	**

ns = no significant, * significant ($P < 0.05$), ** significant ($P < 0.01$)

Table 2: The effect of salinity levels and drought on composition (%) of essential oils (EO) of peppermint

	Salinity		
	NaCl mm		
EO constituent	0	50	100
L-(-)Menthol	41.16	51.65	51.95
Menthone	17.09	9.97	14.25
Menthofuran	11.67	6.37	6.04
Pulegone	5.86	1.97	4.75
Isomenthone	0.03	-	-
Trans-sabinenehydrate	0.06	0.21	0.31
D-limonene	0.25	-	-
trans-β-farnesene	0.01	0.89	-
β-pinene	0.08	0.61	-
Menthyl acetate	0.64	0.34	0.25
Isopulegone	0.05	-	0/25
Terpineol-4	3.38	2.44	0.18
Germacrene D	0.03	-	1.77
α-pinene	-	0.23	-
Piperitone	-	1.13	0.57
γ-Terpinene	0.13	-	0.12
trans -caryophyllene	0.04	-	-
Myircene	-	-	-
Viridiflorol	0.04	-	2.9
α-copaene	0.16	-	3.02
γ-Cadinen	0.05	1.52	0.14

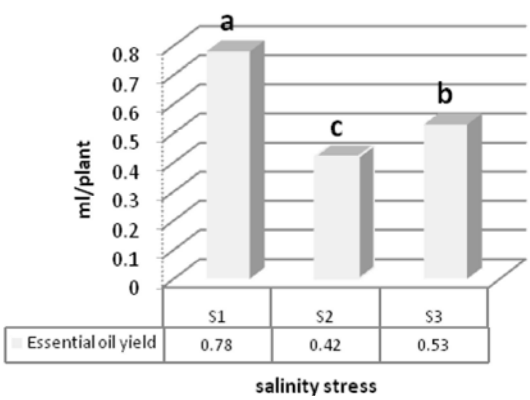

Fig. 1: Essential oil yield under different levels of salinity stress

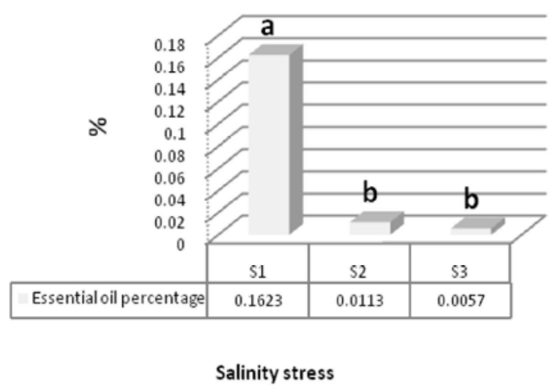

Fig. 2: Essential oil percentage under different salinity stress

the plants. Salinity stress significantly decreased Essential oil yield and Essential oil percent. Olfa *et al.* [1] 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. This result is consistent with those reported by Aziz *et al.* [13] for decreasing in growth parameter and essential oil yield of peppermint grown in different levels of salinity stress. As stated by Munns [14], 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 [15]. Safarnejad [16] showed that increasing salinity stresses caused a reduction, both in shoot and root yield of *Nigella sativa*.

Khammari *et al.* [17] 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+. However, this characteristic was not considered in this study [6]. 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 [8, 10]. As it is shown in Table 3, increase in menthone and the decrease in menthofuran with increasing salinity level improved the commercial quality of the distilled essential oil. This result is consistent with those reported by Charles *et al.* [18] and Tabatabaie for peppermint grown in different levels of osmotic stress [2].

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Funding: Peppermint was moderately tolerant to salinity, because salinity inhibited various growth parameters of this plant to various degrees. Peppermint can be grown successfully on most agricultural soils, as long as NaCl does not exceed the critical values (100 mmol L^{-1} in this study).

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