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Full Length Research Paper

The effect of drought stress on growth parameters, essential oil yield and constituent of Peppermint (*Mentha piperita* L.)

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Peppermint (*Mentha piperita* L.) is one of the most important plants producing essential oil. Two experiments were conducted to determine the effect of drought stress on growth parameters, essential oil constituents and yield of peppermint. 5 levels of water deficit stress including D1 (100% field capacity-control), D2 (85% field capacity), D3 (70% field capacity), D4 (60% field capacity) and D5 (45% field capacity) were investigated during 4 months. Results indicated that drought stress motivated a significant reduction in all of the growth parameters and essential oil yield and percent. The highest values of growth parameters and essential oil percent and yield were observed under 100% field capacity (control). Also, the highest values of Mentone and menthofuran were obtained under 100% field capacity (control) and the highest values of menthol were obtained under 70% field capacity by using gas chromatography-mass spectroscopy (GC-MS).

Key words: Peppermint, essential oil, drought, gas chromatography-mass spectroscopy (GC-MS).

INTRODUCTION

Recently, medicinal and aromatic plants have received much attention in several fields such as agroalimentary, perfumes, pharmaceutical industries and natural cosmetic products (Olfa et al., 2009). Although, secondary metabolites in the medicinal and aromatic plants impressed conventionally by their genotypes, their biosynthesis is strongly influenced by environmental factors too (Yazdani et al., 2002). It means biotic and abiotic environmental factors affect growth parameter, essential oil yield and constituents (Clark and Menary, 2008; Aziz et al., 2008). Abiotic environmental stresses especially salinity and drought has the most effect on medicinal plants (Heidari et al., 2008). Different studies were focused on the effects of salinity 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) and essential oil yield in Lemon Balm (Ozturk et al., 2004). On the other hand, findings of the previous researchers about the effects of drought stress are contradictory. Razmjoo et al. (2008) found that this stress in chamomile reduced some growth parameters and essential oil yield. However, in other studies, it was shown that some growth parameters in basil (Grattan and Grieve, 1999) as well as essential oil amount in Lemon balm (Ozturk et al., 2004) increased as drought stress was increased. In accordance with the results of Farahani et al. (2009), the most essential oil yield in balm was at 60% field capacity (FC). Aziz et al. (2008b) also reported the highest amount of thymol in essential oil at every 10 days plant irrigation. Peppermint (*Mentha piperita* L.) belongs to mint (Lamiaceae) family. It is an herbaceous

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and perennial plant. Also, it is considered as a medical and aromatic plant and extensively using for the medicinal and food product industries (Yazdani et al., 2002; Grieve, 1999). Fundamental components of peppermint essential oil include menthol, menthone, methylacetat, menthofuran and pulegone (Tabatabaie and Nazari, 2007; Mahmoud and Croteau, 2003).

It is necessary to mention that the amount of peppermint essential oil and its constituents considerably were influenced by different factors such as climate, soil type, geographical area (Yazdani et al., 2002) harvest time and fertilizer usage (Niakan et al., 2004; Tabatabaie and Nazari, 2007; Heidari et al., 2008). In this study, the effect of salinity and drought stresses are 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

Peppermint plants were initiated from rhizome cuttings (10 cm long) supplied by Jahad Daneshgahi in March 2009. According to the method described by Farahani *et al*., (2009), seventy five peppermint samples were notified above were transferred into pots (25 cm head diameter × 20 cm height) filled with sandy loam soil (soil average amount of 5580 gr in each pot). This experiment was carried out using a randomized complete block design with 3 replications. The factors studied included 5 levels of water deficit stress that were D1 (100% field capacity), D2 (85% field capacity), D3 (70% field capacity), D4 (60% field capacity) and D5 (45% field capacity), respectively. In order to determine the soil moisture rate of 100% field capacity, 24 h after irrigation we selected 5 field soil samples by sampling drill, then samples were weighed by electrical scale and placed under 105°C in electrical oven for 48 h. Soil moisture rate of 100% field capacity was determined by the following formula.

$$\theta w = \frac{\text{Moist soil weight - Dry soil weight}}{\text{Dry soil weight}}$$

Field soil samples were selected for determination of soil moisture rate daily, than determined 85% field capacity, 70% field capacity, 60% field capacity and 45% field capacity, respectively (Safikhani, 2007; Farahani et al., 2009).

Measurements and statistical analysis

At the end of the experiment (in July 2009), 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 (100 g) in modified Clevenger apparatus (Ozturk et al., 2004). After 3 h 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, 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

The results showed that drought stress significantly affected growth parameters, essential oil yield and constituents of peppermint in $p < 0.05$ (Table 1). Highest shoot fresh weight, shoot dry weight, root dry weight, internodes length, shoot to root ratio, biomass, essential oil yield (Figures 1 to 4) and essential oil percent (Figure 5) were achieved under D1 treatment (control) and highest stem length, root length and stolon length were achieved under D1 and D2 treatments. Also, menthone and menthol, together, accounted for approximately 38.07 - 60.62% of total oil com-position in each treatment. The highest and lowest proportion of Mentone was observed under D1 and D5 treatments, respectively (Table 2).

DISCUSSION

As it was shown in the results of this study, drought stress had a negative effect on most of the growth para-meters under study. As drought level increased from 100-45% FC, shoot fresh weight, shoot dry weight, root dry weight, internodes length, shoot to root ratio, biomass were reduced. Khalid (2006) reported that drought reduced fresh and dry weights in two species of an herb plant that is, *O. basilicum L*. (sweet basil) and *Ocimum americanum* L. (American basil). Aziz et al. (2008b) showed that increasing drought stresses caused a re-duction both in fresh and dry weight of *Deracocephalum moldavica* L. With increasing in drought level, essential oil yield was decreased (Aziz et al., 2008b). Similar results were observed for *D. moldavica* under drought by Hassani (2006). The reduction in essential oil content may be due to disturbance in photosynthesis and carbohydrate production under stress condition and suppression of the plant growth (Flexas and Medrano, 2002). Reduction in oil content as a consequence of drought has also been described in Chamomile (Razmjooo et al., 2008). In *O. basilicum* L. (sweet basil) and *O. americanum* L. (American basil), Khalid (2006) observed that water stress strongly depressed essential oil percentage. How-ever, Ozturk (2004); Safikhani (2007); Farahani (2009) believed that depending upon

Table 1. Effect of salinity levels on growth parameters of Peppermint. The plants died at highest stress dosage (150 mmol L-1 NaCl)

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 percent	Essential oil yield (ml/plant)
0(S1) SE	80a±5.76	31.66a±4.32	63a±6.55	29.66a±3.98	11.86a±1.98	9.23a±1.96	4.33a±1.03	51.38a±3.87	3.1a±0.23	21.09a±3.05	0.78a±0.06	0.1623a±0.03
50(S2) SE	40b±3.43	22a±1.32	6.56b±0.97	3.63b±0.09	1.63b±0.86	1.2b±0.06	2b±0.81	27.66ab±1.98	2.29a±0.02	2.72b±0.04	0.42c±0.02	0.0113b±0
100(S3) SE	42b±2.52	14.66b±2.14	8b±0.64	3.2b±0.38	0.8b±076	3.7b±0.93	1.33b±0.06	3.46b±0.04	2.04a±0.03	1.11b±0.02	0.53b±0.04	0.0057b±0.005
Significance	**	**	**	**	**	*	**	*	ns	**	**	**

Means within the same column and factors, followed by the same letter are not significantly difference (P < 0.05) using Duncan' s. A indicates the top of each values series and b, c etc. indicate the significant lower values respectively. Multiple range test. * Significant (P < 0.05), ** significant (P < 0.01).

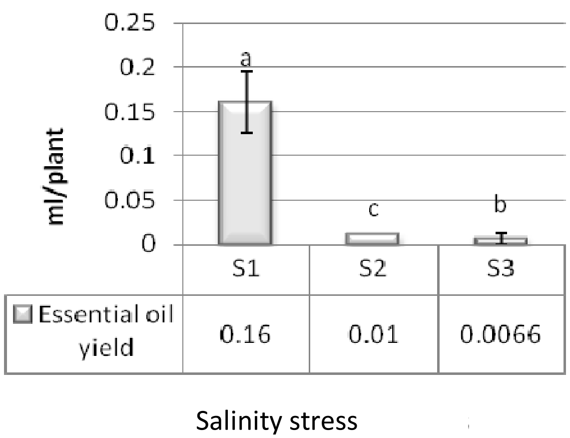

Figure 1. Essential oil yield under different levels of salinity.

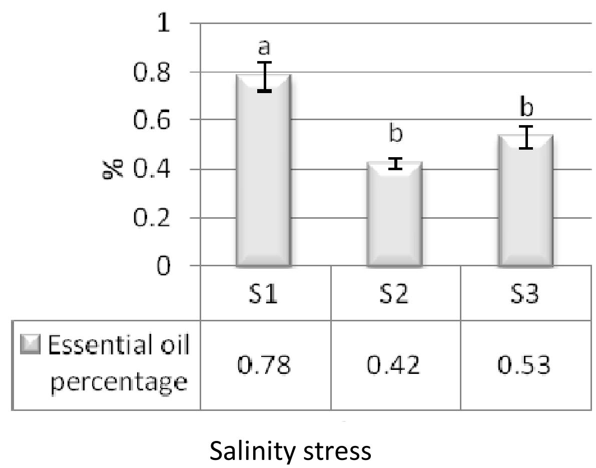

Figure 2. Essential oil percentage under different stress levels of salinity stress.

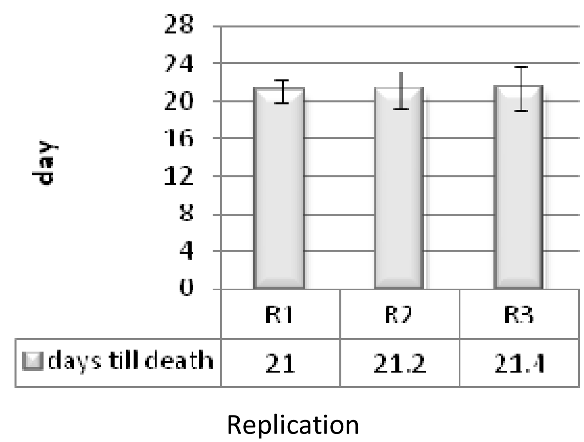

Figure 3. Duration till death of plants related to salinity stress of 150 mmol L-1 NaCl.

the plant species and plant genotype, drought stress can increase, de-crease or have no effect on the levels of metabolites. In this experiment, only one accession was used but based on growth retardation under drought stress conditions it seems that irrigation at optimum condition may promote greater essential oil biosynthesis in peppermint. From a qualitative point of view, the decrease in menthone with increasing drought level increased the commercial quality of the distilled essential oil.

This result is consistent with those reported by

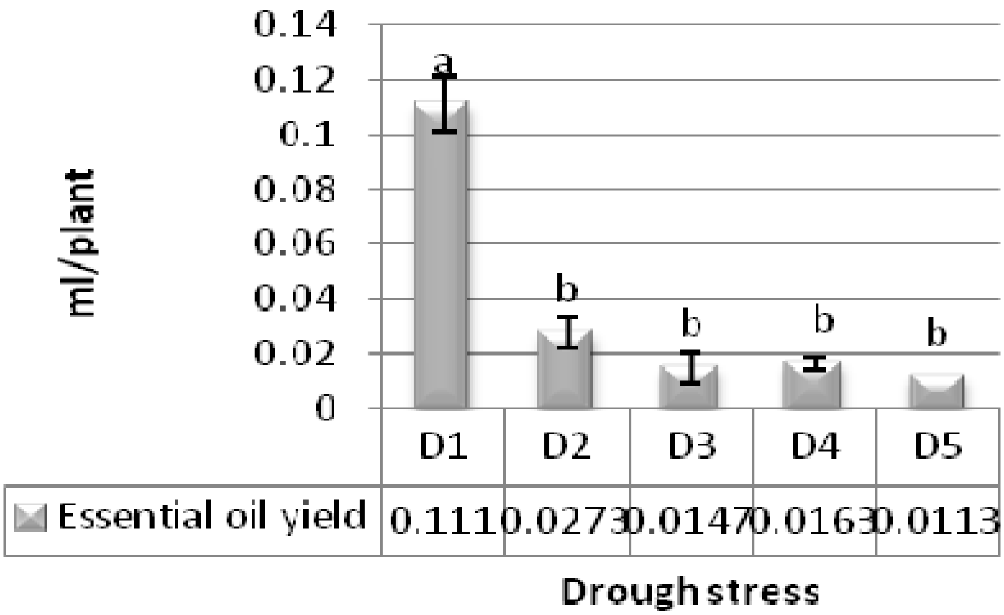

Figure 4. Essential oil yield under different levels of drought.

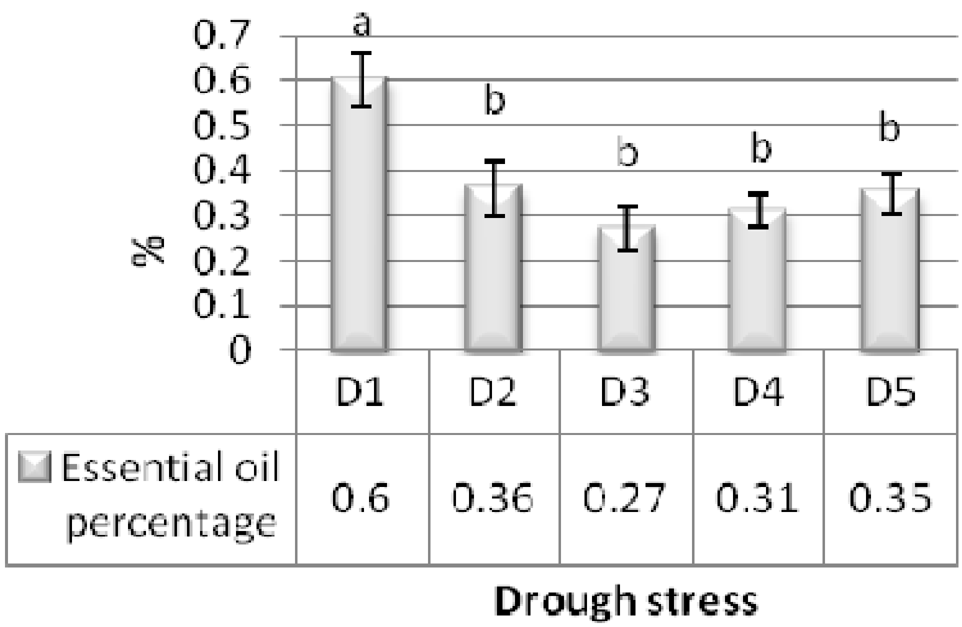

Figure 5. Essential oil percentage under different stress levels of drought stress.

Tabatabaie and Nazari (2007) for peppermint grown in different levels of osmotic stress.

Conclusion

Peppermint was moderately tolerant to water stress, because they did not have negative effects on some growth parameters of this plant drought level of 85% FC may not severely affect growth parameters and oil content of peppermint. Finally, peppermint cannot be grown in soils where enough irrigation water is not available.

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

EO constituent	Salinity NaCl mm				Drought % Fc					
	Significance	0	50	100	Significance	100%	85%	70%	60%	45%
L-(-)Menthol SE	**	41.16±0.51	51.65±6.5	51.95±4.9	**	50.11±6.09	43.36±4.01	58.03±6.09	56.91±6.47	32.72±4.75
Menthone SE	**	17.09±2.09	9.97±0.92	14.25±2.08	**	10.51±1.23	8.36±0.1	7.67±0.89	7.83±0.89	5.35±0.46
Menthofuran SE	**	11.67±2.98	6.37±0.58	6.04±0.54	**	6.35±0.65	3.7±0.43	3.58±0.43	5.3±0.68	2.22±0.34
Pulegone SE	**	5.86±0.49	1.97±0.15	4.75±0.51	**	0.9±0.43	2.49±0.31	3.07±0.34	4.24±0.38	2.25±0.21
Isomenthone SE		0.03	-	-	ns	-	-	0.01±0.002	0.04±0.00	-
Trans-sabinenehydrate SE	ns	0.06±0.00	0.21±0.03	0.31±0.4	**	0.23±0.03	0.26±0.02	0.2±0.03	0.04±0.03	-
D-limonene SE		0.25	-	-	ns	-	-	0.17±0.02	0.02±0.00	-
Trans-β-farnesene SE	**	0.01±0.001	0.89±0.07	-	*	-	0.6±0.07	0.27±0.03	0.11±0.02	-
β-pinene	*	0.08	0.61	-	**	0.64±0.07	0.37±0.03	0.56±0.46	0.01±0.001	-
Menthyl acetate SE	**	0.64±0.07	0.34±0.05	0.25±0.01	*	0.18±0.02	0.1±0.002	0.3±0.02	0.18±0.02	2.49±0.38
Isopulegone SE	ns	0.05±0.003	-	0/25±0.03	**	-	-	0.09±	0.05±	-
Terpineol-4 SE	**	3.38±0.34	2.44±0.21	0.18±0.01	*	1.89±0.13	1.9±0.23	2.3±0.21	1.8±0.21	1.24±0.11
Germacrene D SE	*	0.03±0.002	-	1.77±0.16	**	2.23±0.29	0.79±0.08	0.33±0.04	0.04±0.004	1.1±0.21
α-pinene SE		-	0.23	-	**	0.22±0.01	0.18±0.02	0.17±0.01	0.12±0.01	-
Piperitone SE	**	-	1.13±0.21	0.57±0.04	**	2.2±0.37	1±0.15	-	-	-
Terpinene- γ SE	**	0.13±0.02	-	0.12±0.01	*	0.15±0.01	-	-	0.05±0.002	-
Trans–caryophyllene SE	*	0.04	-	-	**	-	-	-	0.11	-
Myircene SE		-	-	-	*	0.06±0.001	0.2±0.03	0.13±0.01	0.16±0.01	-
Viridiflorol SE	*	0.04±	-	2.9±	**	0.14±	0.13±	-	0.03±	0.35±
-Copaeneα SE	**	0.16±0.01	-	3.02±0.31	**	-	0.19±0.01	-	0.02±0.003	-
Cadinen-γ SE	*	0.05±0.003	1.52±0.01	0.14±0.01	ns	2.45±0.21	1.76±0.21	0.09±0.00	0.15±0.02	0.8±0.06

helping and continuous encouragement to carry out the investigation.

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