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Growth and essential oil of *Mentha longifolia* L. (var. amphilema) from different ecological conditions

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ABSTRACT

Evaluating the adaptability and field longevity of medicinal plants is important for their domestication and economical production, especially in areas with harsh environmental conditions where many crops fail to grow. In this agroecological comparison, samples of Mentha longifolia were collected from two natural habitats in Iran, from 1999-2004. Then, samples were planted at the Research Institute of Forests and Rangelands, Tehran, Iran, and were evaluated during six years. Results indicated that the two populations varied in their essential oils content and composition. Moreover, all the measured traits were significantly different during the different years. Population one had higher leaf and flowering shoot essential oil yield and flowering shoot yield. Among the years, plant height (111.63 cm) and the number of lateral branches (36.66) were the highest in the first year. However, the total essential oil yield (21.51 kg/ha) and flowering shoot yield (3029.66 kg/ha) were the highest in the fourth year. GC and GC-MS analysis detected carvone as the main compound in both populations (72.3% in population one and 62.3% in population two). Results of this experiment briefly indicated that M. longifolia produces the highest biomass and essential oils yield up to the forth year.

Keywords: adaptation, carvone, essential oil, natural habitat.

INTRODUCTION

Environmental conditions are important factors affecting plant growth and yield [2]. As an instant, Voirin *et al*. [4] reported that light and day length affected plants essential oil content. Other studies have also reported the influence of mineral nutrients [13], drought [14], light intensity and altitude [12] on plants growth and essential oil content.

Laminaceae is a plant family widely distributed around the world. This family comprises about 200 genera and 2000-5000 aromatic woody species [1, 9]. Six species of *Mentha* genus are reported to grow in Iran [17]. *Mentha* is a medicinal plant that helps to cure microbial [8] and non microbial [5] diseases. Moreover, *Mentha* essential oils inhibit activity of bacteria [15] and fungi [6].

Species of the *Mentha* genus grow in different habitats, so they are adapted to variety of environmental conditions. Saber Amoli *et al*. [16] described the effects of altitude, topography, soil type and other factors on mint. Mirzaie-Nodoushan *et al*. [7] studied the variations in two clones of *M. longifolia* L. var. amphilema and reported different plant height (121 and 78.33 cm), leaf length (6.03 and 5.13 cm), stem diameter (7.57 and 6.87 mm), flower essential oil content (1.75 and 2.24%) and leaf essential oil content (1.77 and 1.85%). Generally, it can be concluded that environmental conditions greatly affect plant growth and essential oils content and composition. So this experiment

was conducted to compare the growth and essential oils content and composition of two *Mentha longifolia* L. var. amphilema populations collected from different environmental conditions and cultivated in Tehran.

MATERIALS AND METHODS

This agroecological evaluation was conducted during 1999-2004, at the Research Institute of Forests and Rangelands, Tehran, Iran, to compare two *Mentha longifolia* L. var. amphilema populations collected from different habitats. The experiment was conducted in split plot in time in the form of a randomized complete block design with three replications. The main factor was population and the sub factor was year.

In spring 1998, samples were collected from two natural habitats; the long term climatic information of the two habitats is below:

Habitat 1 (Ghazvin province). Latitude, 36^{o} 15' N; longitude, 50^{o} 3' E; elevation, 1279 m above the sea level; mean air temperature, 14.3^{o}C; annual precipitation, 316 mm; relative humidity, 51%.

Habitat 2 (Ardebil province). Latitude, 38^{o} 15' N; longitude, 48^{o} 17' E; elevation, 1332 m above the sea level; mean air temperature, 9^{o}C; annual precipitation, 304 mm; relative humidity, 71%.

Although the two collected populations were morphologically different, they were both identified as *M. longifolia* L. var. amphilema by experts of the Research Institute of Forests and Rangelands, Iran. After identification and propagation by stem cutting, *Mentha* was planted in spring 1998 based on 16 plants/m^2 density, with an interspace of 25 cm. The soil type at the test site was loamy at 0-15 cm and sandy clay loam at 15-30 cm. Other properties of the soil are listed in Table 1. Climatic information of the experimental field (in Tehran province) is also listed in Table 2.

In all six years of the experiment, morphological features of plants were evaluated at the full flowering stage. To study the flowering shoot yield, inflorescence yield, leaf yield, inflorescence essential oil yield and flowering shoot essential oil yield, samples were harvested from 3-5 cm above the soil surface and were dried under shadow and open air flow. When the samples were dried, a small portion of each sample was dried again in a 75°C oven for 24 h to obtain the moisture of open air dried samples. This was to reach the real dry weight of samples. Then, the essential oils were extracted by the method of hydrodistillation using a Clevenger for 2 h. Samples were then dehydrated by sodium sulfate. Finally, regarding the moisture percentage of the dried samples, essential oil yield and content was calculated.

Harvest was conducted two times a year. Morphological features and the essential oil content were represented based on the mean of the two harvests; however, flowering shoot yield was represented based on the sum of the two harvests.

To sustain soil fertility during the six no-till years of the experiment, 40 kg urea/ha was applied annually. The fertilizer was split in two parts; one was added at the beginning of growing season and another was added after the first harvest.

GC and GC-MS were used to detect the main compounds in essential oils [10].

The properties and methods of GC analysis. GC analysis was carried out using Shimadzu GC-9A gas chromatograph equipped with DB-5 column (60 m × 0.25 mm × 0.25 µm). The temperature was kept 50°C for the first 5 min and was programmed to increase up to 250°C at the rate of 4°C/min. Injector and detector temperature was 260°C, the carrier gas was helium with linear velocity of 32 cm/s.

The properties and methods of GC-MS analysis. GC-MS analysis was conducted on a Varian 3400 GC-MS system equipped with a DB-5 column (60 m × 0.25 mm × 0.25 µm). The temperature programming was similar to GC. Carrier gas was helium with linear velocity of 31.5 cm/s; scan time, 1 s; ionization energy, 70 V; and mass range, 40-340 amu.

SAS software was used for data analysis and mean comparison was conducted according to the Duncan's multiple range test.

Table 1. Properties of the test site soil

Depth (cm)	pH	EC (ds/m)	Ca (%)	N (%)	C (%)	Na (mg/kg)	P (mg/kg)	K (mg/kg)	Clay (%)	Silt (%)	Sand (%)	Class
0-15	8.5	0.22	3.1	0.04	0.57	38.7	10.2	197.6	25	30	45	L
15-30	8.4	0.19	3.6	0.04	0.68	32.2	8.7	178.6	21	26	53	Sa.C.L

Table 2. Climatic information of the experimental field during the six years of the experiment

Month	Year 1 (1999)			Year 2 (2000)			Year 3 (2001)			Year 4 (2002)			Year 5 (2003)			Year 6 (2004)		
	Mean Tepm. (°C)	Mean precipitation		Mean Tepm. (°C)	Mean precipitation		Mean Tepm. (°C)	Mean precipitation		Mean Tepm. (°C)	Mean precipitation		Mean Tepm. (°C)	Mean precipitation		Mean Tepm. (°C)	Mean precipitation	
		Rain (mm)	Snow (cm)		Rain (mm)	Snow (cm)		Rain (mm)	Snow (cm)		Rain (mm)	Snow (cm)		Rain (mm)	Snow (cm)		Rain (mm)	Snow (cm)
January	3.4	37.4	2.0	2.7	34.7	4.0	2.3	22.7	80	2.3	23.9	110	4.3	11.6	10.0	4.7	43.8	12.0
February	7.8	7.9	1.0	4.0	32.0	15.0	5.3	8.3	14	6.1	6.0	4.0	4.45	35.2	13.0	7.3	8.0	0
March	8.8	27.5	0	8.4	15.0	2.0	10.3	29.0	0	10.9	22.4	0	7.9	47.2	0	11.1	29.6	15
April	14.8	3.4	0	17.5	3.9	0	17.2	1.0	0	12.8	76.0	0	13.4	63.3	0	12.8	51.4	0
May	20.6	1.3	0	21.3	0	0	20.4	21.0	0	18.7	14.7	0	17.8	18.4	0	18.2	14.6	0
June	25.1	0	0	24.5	0	0	23.3	1.2	0	24.7	0	0	23.4	0	0	25.4	0	0
July	25.4	18	0	28	0	0	26.2	0.8	0	27.3	3.0	0	17.8	0	0	25.8	12.5	0
August	28.3	0	0	27.8	0	0	27.7	3.8	0	27.4	0	0	26.8	0.4	0	27.6	0	0
September	22.2	2.0	0	23.7	1.0	0	23.5	1.8	0	25.1	0	0	22.2	0	0	22.5	0	0
October	17.9	17.4	0	15.2	53.6	0	17.3	18.0	0	20.1	3.3	0	19.8	21.2	0	16.9	4.0	0
November	8.0	43.2	14.0	7.8	21.8	0	10.1	26.0	2.0	10.4	27.9	0	9.1	20.2	0	9.8	40.4	0
December	5.3	25.4	19.0	4.6	78	7	7.4	40.0	0	1.8	83.8	41.0	4.1	35.7	1.8	2.2	33	9

Table 3. Analysis of the variances of the measured traits

SOV	df	Mean Square (MS)												
		Plant height	Leaf length	Leaf width	Stem diameter	Lateral shoot	Flower yield	E.O.P. of flower	E.O. yield of flower	Leaf yield	E.O.P. of leaf	E.O. yield of leaf	Total E.O. yield	Yield of flowering shoot
Replication	2	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Main plot (population)	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*	*
Main error	2	70.786	0.165	0.093	0.147	2.777	323694926	0.006	73534.58	4866458754.2	0.004	1243712.4	852007.7	2728.665
Sub plot (year)	5	**	**	**	**	**	**	**	**	**	*	**	**	**
Population × Year	5	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Rep × sub plot	10	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error	10	14.532	0.474	0.0759	0.389	10.111	464709313	0.006	128749.9	255767688.5	0.008	735702.4	1591570.3	8491.544
CV (%)	-	5.164	18.884	14.934	17.5	10.985	7.429	7.925	11.635	4.953	6.631	9.394	7.266	3.534

*ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.*
E, Essential; O, Oil; P, Percentage.

Table 4. Variations in the measured traits in different years

year	Plant Height (cm)	Leaf length (cm)	Leaf width (cm)	Stem diameter (mm)	Lateral shoot (N/plant)	Flower yield (kg/ha)	E.O.P. of flower (%)	E.O. yield of flower (kg/ha)	Leaf yield (kg/ha)	E.O.P. of leaf (%)	E.O. yield of leaf (kg/ha)	Total E.O. yield (kg/ha)	Yield of flowering shoot (ton/ha)
Year1	111.633a ±10.44SD	5.58a ±0.67SD	2.73a ±0.2SD	7.22a ±0.63SD	36.66a ±2.06SD	263.40c ±14.33SD	0.85b ±0.05SD	2.244c ±0.25SD	935.886d ±43.57SD	1.39a ±0.10SD	13.326c ±1.55SD	15.583c ±1.6SD	2.434d ±0.69SD
Year2	85.167b ±2.73SD	4.66b ±1.36SD	2.28b ±0.44SD	4.17b ±0.99SD	32.66ab ±4.5SD	318.50a ±15.67SD	0.95b ±0.07SD	3.022b ±0.23SD	1008.595cd ±32.61SD	1.48a ±0.14SD	15.036bc ±1.79SD	18.059b±1.85SD	2.722C ±1.0SD
Year3	74.667c ±6.15SD	3.32c ±0.4SD	1.63c ±0.15SD	3.77b ±0.45SD	32b ±2.6SD	309.38a ±47.18SD	1.14a ±0.10SD	3.620a ±0.4SD	1096.395b ±60.48SD	1.45a ±0.07SD	15.929b ±1.38SD	19.384b±1.36SD	2.868b ±0.62SD
Year4	69.637d ±7.97SD	3.26c ±0.13SD	1.55c ±0.17SD	2.63c ±0.31SD	27.16c ±2.48SD	330.66a ±19.64SD	1.12a ±0.08SD	3.706a ±0.26SD	1254.166a ±56.6SD	1.44a ±0.05SD	18.172a ±1.25SD	21.507a ±1.81SD	3.029a ±1.28SD
Year5	63.75e ±5.57SD	2.8cd ±0.26SD	1.48c ±0.08SD	2cd ±0.08SD	25.66c ±2.16SD	288.59bc ±14.53SD	1.05a ±0.101SD	3.048b ±0.37SD	1035.625bc ±53.16SD	1.43a ±0.05SD	14.82bc ±0.96SD	17.875b±0.82SD	2.710c ±0.71SD
Year6	38.333f ±3.94SD	2.25d ±0.15SD	1.38c ±09SD	1.6d ±0.08SD	19.5d ±1.51SD	230.28d ±9.98SD	0.92b ±0.05SD	2.125c ±0.04SD	775.083e ±37.53SD	1.24b ±0.14SD	9.628d ±1.28SD	11.754d±1.26SD	1.876e ±0.97SSD

Means in a column followed by the same letter are not significantly different at P≤0.01.
E, Essential; O, Oil; P, Percentage.

Table 5. Pearson correlation coefficients of the measured traits (N=12).

	Plant height	Leaf length	Leaf width	Stem diameter	Lateral shoot	Flower yield	E.O.P. of flower	E.O. yield of flower	Leaf yield	E.O.P. of leaf	E.O. yield of leaf	Total E.O. yield	Yield of flowering shoot
Plant height	1												
Leaf length	-0.006ns	1											
Leaf width	-0.125ns	0.94**	1										
Stem diameter	-0.094ns	0.85*	0.95**	1									
Lateral shoot	-0.007ns	0.93**	0.92**	0.90**	1								
Flower yield	-0.068ns	0.91**	0.87*	0.82*	0.87**	1							
E.O.P. of flower	-0.187ns	0.25ns	0.18ns	0.05ns	0.016ns	0.415ns	1						
E.O. yield of flower	-0.057ns	-0.30ns	-0.48ns	-0.63*	-0.48ns	-0.17ns	0.64*	1					
Leaf yield	-0.125ns	0.01ns	0.15ns	-0.32ns	-0.23ns	0.153ns	0.89**	0.90**	1				
E.O.P. of leaf	-0.066ns	0.26ns	0.09ns	-0.06ns	0.006ns	0.306ns	0.87**	0.71**	0.88**	1			
E.O. yield of leaf	-0.421ns	0.47ns	0.40ns	0.25ns	0.21ns	0.526ns	0.73*	0.26ns	0.55ns	0.65*	1		
Total E.O. yield	-0.193ns	0.34ns	0.20ns	0.03ns	0.07ns	0.399ns	0.90**	0.62*	0.85**	0.97**	0.82**	1	
Yield of flowering shoot	-0.200ns	0.29ns	0.15ns	-0.02ns	0.02ns	0.371ns	0.92**	0.68*	0.89**	0.97**	0.80**	0.99*	1.00

*ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.*
E, Essential; O, Oil; P, Percentage.

RESULTS

Results indicated that leaf essential oil content, flowering shoot essential oil and flowering shoot yield were significantly different between the two populations (P≤0.05; Table 3). The effect of year was also significant on all measured traits (P≤0.01). The interaction of population × year had only a significant effect on plant height (P≤0.01); the effect was not significant on rest of the measured traits. Mean comparison indicated that population 1 (Ghazvin) had higher leaf essential oil yield (15.20 kg/ha), flowering shoot essential oil yield (18.002 kg/ha) and flowering shoot yield (2644.2 kg/ha) compared with population 2 (Ardebil), which gave 13.953 kg/ha leaf essential oil content, 16.12 kg/ha flowering shoot essential oil yield and 2570.03 kg/ha flowering shoot yield.

Among the six years of the experiment, plant height (111.63 cm), leaf length (5.58 cm), leaf width (2.73 cm) and stem diameter (7.22 cm) were the highest in the first year, and leaf yield (1254.17 kg/ha), leaf essential oil yield (18.17 kg/ha), total essential oil yield (21.51 kg/ha) and flowering shoot yield (3029.33 kg/ha) were the highest in the fourth year (Table 4).

Flowering shoot yield was significantly correlated to inflorescence essential oil content and yield, leaf yield, leaf essential oil content and yield and the total essential oil yield (Table 5). The total essential oil yield was also significantly correlated to inflorescence essential oil content and yield, leaf yield and leaf essential oil content and yield. Plant height which gradually reduced from the first to the sixth year was not significantly correlated to any other measured trait.

Results of essential oil analysis by GC and GC-MS indicated that compounds varied greatly in two populations. The three dominant compounds in Ghazvin population were carvone (72.3%), limonene (19.29%) and β-gurjunene (1.33%); however, in Ardebil population were carvone (62.3%), 1, 8-cineole (14.31%) and neo-isomenthol (4.98%). Moreover, 13 compounds were only detected in Ghazvin population, and five other compounds were only detected in Ardebil population (Table 6).

Table 6. Essential oil composition of the two *Mentha longifolia* L. var. amphilema populations

Retention Time	Retention Index	Compounds	Content (%)	
			Population 1	Population 1
10.0333	944	α-pinene	0.98	0.52
11.2667	972	sabinene	0.58	1.09
11.4333	976	β-pinene	1.04	0.95
11.8333	986	Myrcene	0.96	0.48
12.7167	1011	α-terpinene	-	1.61
13.3333	1019	1,8-cineole	-	14.31
13.5333	1021	Limonene	19.29	0.11
14.55	1074	γ - terpinene	-	0.34
17.5333	1129	Trans pino carveol	-	1.29
18.3833	1153	pinocarveone	0.7	0.6
18.9333	1158	Terpin-4-ol	0.9	-
19.2833	1172	isomenthole	-	1.77
19.3133	1179	Neo-iso menthol	1.13	4.98
20.3667	1203	iso-dihydro carveol	0.12	-
20.4833	1207	Trans -carveol	0.53	-
21.0167	1219	carvone	72.3	62.3
21.4167	1223	piperitone oxide	-	0.4
21.5333	1221	Piperitone	-	.39
21.4554	1281	linalool	0.32	-
24.3333	1309	Neo- verbanol acetate	-	0.73
25.1167	1331	Terpinene-4-ol acetate	-	0.3
26.9333	1383	β-elemne	-	0.83
28.1167	1417	isobornyl isobutyrate	-	2.59
28.2667	1423	β-gurjunene	1.33	0.11
29.6833	1463	α-terpinyl iso butyrate	-	0.14
33.0333	1568	sapthulenol	-	0.13
36.1833	1677	α-bisabool	0.13	-
36.3	1683	germacrone	0.6	0.6

DISCUSSION

Results indicated that the highest plant height, leaf length and width, stem diameter and the number of lateral branches were achieved in the first year. The highest inflorescence yield, leaf yield and flowering shoot yield were achieved in the fourth year and reduced in the following years. Essential oil yield had an increasing trend up to the fourth year; however, reduced in fifth and sixth year. Better soil physical condition and lower compaction in the first

years of the experiment, and climatic conditions describe why morphological traits were superior in the first years. In the fourth, fifth and sixth years, high snow and low temperature at early spring (Table 2) inhibited vegetative growth and weakened morphological characteristics. The increased flowering shoot yield in the fourth year can be attributed to the increased number of rhizomes and stems. Moreover, the increased essential oil yield may be attributed to the low diameter of the main stem because main stem usually contains very low essential oils.

The significant difference of flowering shoot yield and essential oil yield between the two populations may be attributed to the increased number of sucker, because other traits were not significantly different. No significant difference in leaves essential oil content, which is the main trait affecting leaf essential oil yield and flowering shoot yield, indicates that flowering shoot yield is an important factor for the selection of suitable population.

Correlations indicated that dry leaf and flower weight had the highest effect on the improvement of flowering shoot yield. It can be concluded from the correlations that populations with longer leaves produce wider leaves, ticker flowering shoot branches and higher number of lateral branches. Higher inflorescence essential oil content resulted in higher leaf essential oil content. Moreover, higher number of leaves resulted in the enhancement of essential oil content.

GC and GC-MS analysis revealed that carvone was the dominant compound in essential oils of both populations; however, the content was different in the two populations. This is probably controlled by genetic factors. The environmental conditions of parent plants' growth habitat such as light, soil type, available water and temperature have also effect on the composition and content of essential oil. Other studies also indicated that soil nutrient content had effect on the composition of essential oil in balm (*Melissa officinalis* L.) and peppermint (*Mentha piperita* L.) [3, 11]. Finally, as the essential oil is the main product of *Mentha*, it seems that this plant gives an economical yield up to four years.

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