Turkish Journal of Agriculture and Forestry

Volume 26 | Number 4 Article 7

1-1-2002

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Turk J Agric For
26 (2002) 217-224

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Received: 15.10.2001

Abstract: Eleven lemon balm (*Melissa officinalis* L.) populations originating from different sources in Turkey and European countries were investigated in two ecologically different locations, Menemen and Bozdağ, in the Aegean region of Turkey over three years to determine the populations having high quality and yield. There were significant variations between locations, years and populations in terms of yield and quality characters. The ecology was highly suitable in Menemen for growing lemon balm successfully and almost all yield and quality characters in Menemen were significantly higher than those in Bozdağ. The plant height, green herb yield, drug leaves yield and essential oil rate over populations and years were 47.58 cm, 2869 kg.ha^{-1}, 496.9 kg.ha^{-1} and 0.067% respectively in Menemen while they were 20.73 cm, 416 kg.ha^{-1}, 90.0 kg.ha^{-1} and 0.036% respectively in Bozdağ. The growth of populations in this study increased after the first year of the trials in both locations; therefore all yields were significantly higher in the second and third years of the trials compared to the first year. The populations numbered 9 and 7, originating from Germany and Romania, had high yield and quality and they were the two most promising populations for lemon balm cultivation in the locations studied. The green herb yield, drug leaves yield and essential oil rate of population 9 over three years at Menemen were 3389 kg.ha^{-1}, 593.1 kg.ha^{-1} and 0.085% respectively while they were 2888 kg.ha^{-1}, 565.1 kg.ha^{-1} and 0.097% respectively for population 7. The main component of the essential oil of the lemon balm was geranial in both locations and the average geranial rate was 38.13% in Menemen and 53.68% in Bozdağ. Hence, Menemen ecologic conditions were suitable for growing lemon balm with high yield and quality. Populations originating from Romania and Ege University Agricultural Faculty (Germany) are recommended to growers.

Key Words: *Melissa officinalis*, balm, essential oil, green herb, dried leaves, geranial.

Ege Bölgesi'nde Yetiştirilen Oğulotunun Verim Özellikleri ve Uçucu Yağının Bileşimi

Özet: Yüksek verim ve kalite özelliklerine sahip, tarıma uygun oğulotu (*Melissa officinalis* L.) populasyonlarını belirlemek amacı ile yurt içi ve yurt dışı değişik kaynaklardan olmak üzere 11 oğulotu populasyonu Ege Bölgesinde birbirinden farklı ekolojik özelliklere sahip Menemen ve Bozdağ lokasyonlarında üç yıl süre ile adaptasyon denemesine alındı. İncelenen verim ve kalite kriterleri açısından lokasyonlar, yıllar ve populasyonlar arasında önemli varyasyonlar tespit edilmiştir. Menemen ekolojisinin oğulotu yetiştiriciliği açısından oldukça uygun olduğu saptanmış ve bütün verim ve kalite kriterleri Menemen'de Bozdağ'dakilerden daha yüksek bulunmuştur. Bitki boyu, yeşil herba verimi, drog yaprak verimi ve uçucu yağ oranı populasyonlar ve yıllar üzerinden ortalama değer olarak Menemen'de sırası ile 47,58 cm, 2869 kg.ha^{-1}, 496,9 kg.ha^{-1} ve% 0,067 olurken, Bozdağ'da sırası ile 20,73 cm, 416 kg.ha^{-1}, 90,0 kg.ha^{-1} ve % 0,036 olmuştur. Denemenin birinci yılından sonra bitki gelişmesi dolayısı ile incelenen tüm verim kriterlerinde birinci yıl verilerine göre önemli derecede artış görülmüştür. Bu araştırmada 9 ve 7 numaraları verilen sırası ile Almanya ve Romanya orijinli iki populasyon tüm verim ve kalite kriterleri açısından her iki lokasyonda da denemeye alınan diğer populasyonlardan daha üstün performans göstermişler ve başarılı bir şekilde tarımı yapılabilecek ümitvar populasyonlar olarak saptanmışlardır. Populasyondan 9'un Menemen'de yıllar üzerinden yeşil herba verimi, drog yaprak verimi ve uçucu yağ oranı sırası ile 3389 kg.ha^{-1}, 593,1 kg.ha^{-1} ve % 0,085 olarak bulunurken; 7 nolu populasyon için aynı değerler sırası ile 2888 kg.ha^{-1}, 565,1 kg.ha^{-1} ve % 0,097 olarak bulunmuştur. Her iki lokasyonda da uçucu yağın ana bileşeninin geranial olduğu tespit edilmiştir. Yıllar ve populasyonlar üzerinden ortalama değer olarak uçucu yağdaki geranial oranı Menemen'de % 38,13, Bozdağ'da ise % 53,68 olarak saptanmıştır. Sonuç olarak Menemen ekolojik koşullarının verim ve kalitesi yüksek oğulotu yetiştiriciliği için uygun olduğu, özellikle Romanya ve Ege Üniversitesi Ziraat Fakültesi (Almanya) orijinli populasyonların üreticilere önerilebileceği belirlenmiştir.

Introduction

Lemon balm (*Melissa officinalis* L.), a member of *Laminaceae*, is a perennial plant growing up to 100 cm. It has been used for a long time to attract honeybee swarms to hives and the word *Melissa* means bee in Greek (Burgett, 1980; Ceylan, 1997). This species originates

from southern Europe, Asia Minor and southern parts of North America (Simon et al., 1984; Ceylan, 1997). Lemon balm populations are distributed in all Mediterranean countries including the coastal regions of Turkey and southern Alpine regions (Baytop, 1984; Ceylan, 1997). There are three subspecies of *M. officinalis*: subsp. *officinalis*, subsp. *inodora* and subsp. *altissima*; however, only subsp. *officinalis* has commercial value and the characteristic lemony odor of lemon balm (Mill, 1982; Baytop, 1991; Craker and Simon, 1992). Lemon balm is used for several purposes such as an additive in food, a herb tea, an ingredient in cosmetics, an ornamental and a medicine (Simon et al., 1984; Akgül, 1993). This plant has traditionally been used to treat catarrh, fever, flatulence, headaches, influenza and toothaches. It also has sedative, antidepressant, antiviral, antibacterial and antispasmodic effects (Zeybek, 1987; Baytop, 1984 and 1991; Simon et al., 1984; Ceylan, 1997; Tyler, 1999). Because the essential oil rate of lemon balm is quite low, the production cost and price of the oil are very high. Therefore, lemon balm oil is sometimes adulterated with *Cymbopogon* spp. or *Citrus* pile oil. Lemon balm has been cultivated in some European and Balkan countries, but not in Turkey.

The aim of this study was to determine the adaptation capabilities of different lemon balm populations to various ecological conditions and to assess the populations with high yield drug leaves and essential oil contents for initiating cultivation of this plant in Turkey.

Materials and Methods

Materials

The seeds of 11 lemon balm populations obtained from different sources were compared in this study (Table 1). Eight of them originated from European countries and the others were native to Turkey and originated from the Aegean region. Populations 1, 2 and 3 were introduced from Germany having been indicated as wild populations. There was no information about whether populations 4, 5, 6 and 7, introduced from Spain (Cuenca), Spain (Madrid), Hungary and Romania, respectively, were wild or cultivars.

Population 8 was collected by the authors from the foot of Bozdağ at 300 m. Populations 9, 10 and 11 were obtained from Ege University Agricultural Faculty Field Crops Department. Population 9 was a cultivar and had been introduced from Germany by Ege University. Populations 10 and 11 were collected from the Aegean region. Field trials with the 11 populations given above were conducted in two ecologically different locations, Menemen and Bozdağ. Their soil characteristics are presented in Table 2.

Menemen is around sea level at an altitude of 10 m. Therefore, Menemen has mild weather. In contrast, Bozdağ is a high and hilly snowy location at an altitude of 1000 m and has cold weather and severe winters. Precipitation is mainly rain in Menemen and snow in Bozdağ. Yearly average precipitation is 550 mm in

Table 1. Population numbers, accession numbers and origins of lemon balm (*Melissa officinalis* L.) populations.

Population Number	Accession Number	Origin
1	*PGR-PI 7295-94	Germany
2	PGR-PI 7297-94	Germany
3	PGR-PI 7296-94	Germany
4	PGR-PI 7300-94	Spain (Cuenca)
5	PGR-PI 7301-94	Spain (Madrid)
6	PGR-PI 7429-94	Hungary
7	PGR-PI 7530-94	Romania
8	-	Turkey (Ödemiş-Bozdağ)
9	-	**EUAF (Germany)
10	EUAF 844	EUAF
11	EUAF 908	EUAF

*PGR-PI: Plant Genetic Resources-Plant Introduction.

**EUAF: Ege University Agricultural Faculty

Table 2. Structure and chemical composition of soil in trial areas at Menemen and Bozdağ.

Location	Sand	Clay	Silt	Total Salt	$CaCO_3$	Organic Matter	pH	P	K
				(%)				(mg kg^{-1})	
Menemen	51.98	29.16	18.86	0.066	3.6	1.6	7.70	12.23	267
Bozdağ	73.28	4.95	21.77	0.055	0	1.7	5.73	49.64	75.16

Menemen and 1250 mm in Bozdağ. Yearly average air temperature is 16.85 °C in Menemen. Since Bozdağ does not have a weather station, we do not have average yearly temperature records for Bozdağ.

Methods

After growing lemon balm seedlings to around 10 cm in seed beds, adaptation trials of the lemon balm in this study were established in completely randomized block design with four replications on March 28th 1995 in Menemen and May 16th 1995 in Bozdağ. Lemon balm seedlings were transplanted with 45 cm row space and 25 cm apart in the row. The length of each row was 2.5 m and there were four rows in each plot. The plants on the two central rows in each plot were cut when 50% of the lemon balm plants had flowers. Lemon balm plants in the other two rows and 25 cm at the two ends of the harvested rows were left for side effect. The adaptation trials lasted three years in Menemen and two years in Bozdağ. The following agronomic and quality characters of lemon balm populations were recorded and evaluated at the end of the trial.

Plant height (cm): Measured just before every cutting from soil level to highest point with five plants in each plot.

Green herb yield (kg.ha^{-1}): After the side effect was left as mentioned above, the remaining plants in each plot were cut by hand with saw knife 10 cm above the surface and immediately weighed for obtaining plot yield. Then plot yield was transformed to yield for ha^{-1} (1000 square meters).

Drug leaves yield (kg.ha^{-1}): A sample of 500 g of green herb was taken from each plot. Then these samples were dried in an oven at 35 ºC for 72 h. After drying, samples were weighed. Then the leaves of the samples were separated from the stems by hand and weighed. The ratio of dry leaves to green herb was obtained and, therefore, dry leaves yield was calculated.

Essential oil rate (%): A sample of 20 g of drug leaves was taken from each plot. Then 200 ml of tap water was added to each sample and water distillation was run for 45 min using a neo-clevenger apparatus. The essential oil rate was measured by the volumetric method (v/w) (Wichtl, 1971).

Composition of essential oil (%): Components of essential oil was determined by gas chromatography (GC) (2380 model Carlo Erbaa-Fractovap) at Ege University Central Laboratory. Column length was 3 m. Temperatures of injector, column and detector were 250 °C, 110 ºC and 250 ºC, respectively. The material of the column was 3% OV-1 and Gas Chrom Q. Peaks taken from GC were identified using reference solutions of α-pinen, β-pinen, linalool, citronellal, borneol, neral, geraniol and geranial.

The results obtained in both locations were evaluated separately by using the SPSS for Windows computer program according to completely randomized block design for two factors. Years and populations were the factors. Locations were compared by paired t test.

Results

Plant height (cm): There were significant differences among populations for plant height in both locations. Plant height was higher in Menemen than in Bozdağ (Table 3). Plant heights of populations ranged from 41.61 to 55.79 cm in Menemen and from 10.58 to 29.61 cm in Bozdağ. Average plant heights for populations in Menemen and Bozdağ were 47.58 cm and 20.73 cm, respectively.

Green herb yield (kg.ha^{-1}): There were significant variations among populations and between locations for green herb yield. Average green herb yield in Menemen was around six times higher than that in Bozdağ (Table 3). It was 2869 $kg.ha^{-1}$ in Menemen and 416 $kg.ha^{-1}$ in Bozdağ. Green herb yield ranged from 2269 to 3433

Table 3. Plant height, green herb yield, drug leaves yield and essential oil rates of 11 lemon balm (*Melissa officinalis* L.) populations in Menemen and Bozdağ over three years.

Locations	Menemen	Bozdağ	Menemen	Bozdağ	Menemen	Bozdağ	Menemen	Bozdağ
Populations	Plant height (cm)		Green herb yield ($kg.ha^{-1}$)		Drug leaves yield ($kg.ha^{-1}$)		Essential oil rate (%)	
1	44.52cd	17.18d	2730bcd	460bc	503.8bcd	108.7bc	0.029d	0.016bc
2	46.57bcd	15.63d	2714bcd	424bc	488.2bcde	106.6bc	0.082b	0.021bc
3	41.61d	14.15d	2533cd	325c	473.0cde	79.1cd	0.079b	0.020bc
4	47.88bc	26.35ab	2650bcd	688a	522.6abc	154.3a	0.092ab	0.059a
5	46.25bcd	25.84b	2754bcd	435bc	469.4cde	82.6cd	0.076b	0.024bc
6	43.88cd	25.89b	2269d	633a	422.4de	128.0ab	0.076b	0.051a
7	43.54cd	24.45bc	2888abc	563ab	565.1ab	126.7ab	0.097a	0.051a
8	55.79a	29.61a	3050abc	355c	456.6cde	61.4de	0.032d	0.039ab
9	49.84b	21.94c	3389a	471bc	593.1a	103.4bc	0.085ab	0.046a
10	55.13a	16.48d	3146ab	170d	411.3e	27.6ef	0.049c	0.010c
11	47.29bc	10.58e	3433a	54d	560.0ab	11.9f	0.044cd	0.063a
Mean	47.58	20.73	2869	416	496.9	90.0	0.067	0.036
$LSD_{(P < 0.05)}$	5.332	3.347	568.7	151.8	84.24	35.80	0.017	0.025
$t_{0.05, 10}$	13.022*		16.371*		20.351*		3.947*	

* $P < 0.05$

$kg.ha^{-1}$ in Menemen and from 54 to 688 $kg.ha^{-1}$ in Bozdağ.

Drug leaves yield ($kg.ha^{-1}$): Drug leaves yield was also significantly higher in Menemen than in Bozdağ for all populations (Table 3). Drug leaves yield ranged from 411.3 to 593.1 $kg.ha^{-1}$ in Menemen and from 11.9 to 154.3 $kg.ha^{-1}$ in Bozdağ. Average yield was 496.9 $kg.ha^{-1}$ in Menemen and 90.0 $kg.ha^{-1}$ in Bozdağ.

Essential oil rate (%): There were significant differences among populations and between locations for essential oil rates (Table 3). The essential oil rate was significantly higher in Menemen (0.067%) than in Bozdağ (0.036%). The essential oil rate ranged from 0.029 to 0.097% in Menemen and from 0.010 to 0.063% in Bozdağ.

Components of essential oil: The rates of the following major components of essential oil were determined in this study: α-pinen, β-pinen, linalool, citronellal, borneol, neral, geraniol and geranial. There were significant variations among populations and between locations for the components of the oil.

Components of essential oil in Menemen: The major components of the oil were geranial (38.13%) and neral (12.22%) (Table 4). The rates of other components were as follows: β-pinen 11.73%, citronellal 5.86%, geraniol 4.95%, α-pinen 2.86%, linalool 2.74% and borneol 0.62%.

The populations originating from Turkey had higher β-pinen rates than did the other populations. The citral (neral and geranial) rate, providing the lemony scent of the lemon balm, was higher in populations introduced from Europe than that in populations collected from Turkey. Neral + geranial rate in essential oil was over 70% for populations 1 and 7, over 60% for populations 3 and 9, and over 50% for populations 4 and 6.

Components of essential oil in Bozdağ: The rates of essential oil components were quite different among populations in Bozdağ as they were in Menemen and the major component of the oil was geranial for all populations (Table 5). The geranial rate in Bozdağ (53.8%) was higher than that in Menemen (38.13%). The rates of citronellal, neral and geraniol were close to

Table 4. Components of essential oil of lemon balm (*Melissa officinalis* L.) populations in Menemen over three years.

Population	Components of essential oil							
Number	α-pinen	β-pinen	linalool	citronellal	borneol	neral	geraniol	geranial
				(%)				
1	-	2.42	0.40	9.30	-	9.53	3.96	29.37
2	-	1.50	1.41	9.87	1.33	17.45	4.74	58.64
3	-	2.10	1.63	10.03	0.23	26.61	4.22	38.59
4	1.33	2.36	1.22	1.88	-	20.96	23.22	26.41
5	1.67	3.10	3.90	5.34	-	12.60	2.86	42.27
6	2.00	6.66	9.00	1.31	-	9.85	2.94	39.08
7	-	7.85	1.99	5.72	0.29	13.26	5.87	53.65
8	2.28	11.99	0.55	3.16	4.19	4.52	1.88	54.76
9	-	4.94	5.66	6.50	0.21	15.37	4.79	56.49
10	12.82	41.63	2.65	3.97	0.61	-	-	4.52
11	11.38	44.49	1.71	7.44	-	4.28	-	15.67
Mean	2.86	11.73	2.74	5.86	0.62	12.22	4.95	38.13

Table 5. Components of essential oil of lemon balm (*Melissa officinalis* L.) populations in Bozdağ over three years.

Population	Components of essential oil							
Number	α-pinen	β-pinen	linalool	citronellal	borneol	neral	geraniol	geranial
				(%)				
1	-	-	-	-	-	15.35	-	84.65
2	-	-	-	17.33	-	3.25	6.84	60.2
3	-	-	-	13.95	-	19.32	19.75	46.93
4	-	3.05	1.75	14.64	-	9.30	5.57	51.52
5	-	-	-	4.49	-	6.77	7.19	74.07
6	-	-	-	20.29	-	7.62	7.88	50.40
7	-	-	4.25	19.83	-	12.97	14.57	37.69
8	-	-	-	11.42	-	10.94	10.80	56.17
9	1.05	1.75	4.20	10.11	-	12.10	12.14	46.66
10	-	10.72	-	15.62	-	3.95	5.13	59.57
11	-	7.88	-	12.96	-	32.12	-	35.72
Mean	0.13	1.77	1.28	12.91	0	11.48	8.69	53.68

each other and were 12.91%, 11.48% and 8.69%, respectively. The rates of β-pinen, linalool and α-pinen were 1.77%, 1.28% and 0.13% respectively.

Discussion

Almost all yield and quality characters were significantly higher in Menemen than those in Bozdağ. The reason for these differences was probably different environmental conditions, mainly climate and soil properties in the two locations (Table 2). Five populations had adaptation problems to Bozdağ. Populations 1, 2 and 3 did not grow well enough to allow cutting at the first year of the experiment. Most plants of populations 10 and 11 were damaged or died because of cold weather. Özgüven et al. (1995) reported that lemon balm plants were grown prostrate at high altitude because of high light intensity. They also reported some lemon balm

populations had adaptation problems in Adana. Cold weather damage for the lemon balm was observed at Taşova, Tokat, in Turkey (Demir et al., 2000).

Populations 8, 10 and 11 had high plant height and green herb yield at Menemen. This result might be expected since these populations were wild populations distributed in the Aegean region of Turkey. However, they had low drug leaves yield and essential oil rate, because these populations were wild and not bred for high leaves and oil rate.

Population x year interactions were significant for all yield and quality characters at both locations (not presented here). This indicates that different lemon balm populations have different adaptation capabilities to different ecological conditions. However, populations 9 and 7 had satisfactory yield and quality every year at Menemen. The yield and quality values of these two populations were similar to the results obtained by Ceylan et al. (1994a and b), Özgüven et al. (1999), Tınmaz (1999) and Demir et al. (2000). However, green herb yields of populations 9 and 7 were significantly higher than the 435 g/m^2 green herb yield reported by Rey (1996), but significantly lower than the 6.1 kg/m^2 yield obtained by Putievsky et al. (1978). This large difference in green herb yield was probably due to the fact that Rey and Putievsky used too small an area for obtaining their data. As mentioned before, population 9 was a cultivar introduced from Germany. Although we do not have this kind of information for population 7 introduced from Romania, we think that it is also a bred cultivar because of its high yield and quality values compared with the other populations at both locations.

Populations 4 and 6 probably originated from regions having a cold climate since they had high plant height, green herb yield, dry leaves yield and essential oil rate at Bozdağ, although they had lower yield and quality at Menemen compared with the other populations.

Populations 1, 2 and 3, which originated from Germany, were wild populations as we mentioned in the Materials section. These populations had average yield and quality characters at both locations. This result was not surprising since Demir (1975) reported that wild plants had lower yield and quality than did cultivated ones. Population 5, which originated from Spain (Madrid), might also be a wild population since it had similar results to those of populations 1, 2 and 3.

Essential oil rates, 0.067% in Menemen and 0.036% in Bozdağ, were lower than the 0.157% reported by Malik et al. (1972), the 0.068% reported by Özay (1990), the 0.077% and 0.23% reported by Ceylan et al. (1994a and b) and the 0.141% reported by Tınmaz (1999). On the other hand, our results for the oil rate were similar to the 0.1-0.015% oil rate reported by Baytop (1984), the 0.02% reported by Kırımer et al. (1995) and the 0.03-0.47% reported by Özgüven et al. (1999). Meanwhile, the essential oil rates of the populations had significant differences from year to year. For instance, the essential oil rate in Menemen in the third year of the trial was 0.120% while the essential oil rates of populations 7, 2 and 9 were 0.194%, 0.166% and 0.157% respectively. These results suggest that climate has a significant effect on essential oil rate. We know that the essential oil rates of plants are affected by several factors, mainly by environmental conditions (Penka, 1978; Franz et al., 1984; Adzet and et al., 1992b; Sharma et al., 1992; Ceylan, 1995). The significant differences between essential oil rates of populations make it possible to improve a cultivar with a high oil rate. Adzet et al. (1992a) increased the essential oil rate of lemon balm from 0.2-0.3% to 0.5% via selection breeding.

The main components of the oil at both locations were neral and geranial for all populations, except for populations native to Turkey. Citral (geranial + neral) rates, especially geranial rates for most of the populations were higher in Bozdağ, although the other yield and quality characteristics were higher in Menemen. This indicates that ecological conditions have a significant effect on the composition of the essential oil or that populations have genetically quite different plants. These two factors might also have a combined effect. The main component of the native populations was mostly β-pinen. Therefore, native populations lacked the characteristic odor of lemon balm, the lemony scent. There were significant differences between populations for neral and geranial rates. These rates also had significant differences from year to year. Many researchers have reported that the main components of lemon balm are neral and geranial. However, there were significant differences among the rates of those reported components. Neral and geranial rates in the oil were reported respectively as 15% and 14.5% by Hefendehl (1970), 19.6-36.1% and 25.3-47.5% by Tittel et al. (1982), 19.5% and 31.6%

by Werker et al. (1985), 7.195% and 12.99% by Özay (1990), 10.9% and 17.3% by Lawrence (1983) and 30-40% and 50-60% by Ceylan et al. (1994b). Total citral rate (citral a and b = neral+geranial) was also reported in some papers. The neral+geranial rate in the lemon balm oil was reported to be 65.210% by Ceylan et al. (1994a), over 90% by Adzet et al. (1992b), and 61.3% by Burgett (1980). Although all studies discussed above reported that neral and geranial were the main components of the oil, Kırımer et al. (1995) found that the main component of the lemon balm oil they studied was carvacrol (60%). All the significant differences for the components of the oil among the papers discussed above may be due to the use of different genetic material and/or different environmental conditions. The genetic variations among the lemon balm populations for the rate and components of the oil and the significant effect of the environment on these two are known (Clark et al., 1980; Franz et al., 1984; Sharma et al., 1992; Ceylan et al., 1994a; Kırımer et al., 1995; Özgüven et al., 1995).

In conclusion, we found out that Menemen is quite suitable for cultivating lemon balm. Bozdağ does not have good weather for growing lemon balm. Populations 9 and 7 among 11 populations had satisfactory yield and quality and are promising populations for cultivation. Variations within and between populations for yield and quality characters allow us to select individuals with high yield and quality and to improve a cultivar.

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