THE VARIATION OF ESSENTIAL OIL AND CARVACROL CONTENTS OF NATIVE GROWN *Thymbra spicata* var. *spicata* L.

KAZIM MAVİ[1], DURMUŞ ALPASLAN KAYA[2*], MUSA TÜRKMEN[2], FILIZ AYANOĞLU[2]

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay Turkey*

[2]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Hatay Turkey, * dak1976@msn.com*

In the study, it is aimed to create breeding lines of thyme (*Thymbra spicata* L.), which is important both culturally and economically, by selecting a single plant among the plants grown in different regions in Hatay. For this purpose, a genetic pool was created for Thymbra spicata L. plant in the plant samples taken from the locations where the plants are densely grown, and these plants were examined in terms of leaf characteristics, number of oil glands per unit area, oil gud size and essential oil components. Plants were propagated and preserved with cuttings taken from these single plants. In this study, which includes the pre-selection stage, 213 plants from 68 different locations were determined in the province of Hatay. The essential oil ratios of the plants varied between 0.70% and 3.90% and showed a wide variation. The rate of carvacrol, which is the main component of the essential oil of the thyme plant, was between 28.12% and 78.48%. Plants with code number Z14, Z3, Z25, Z38, Z77, Z104, Z35 and Z43 with an essential oil ratio of 3.5% and above and plants with code number Z167, Z165 and Z64 with a high carvacrol ratio were selected to be used in future breeding studies.

Keywords: *Thymbra spicata* L, essential oil, carvacrol content

INTRODUCTION

Turkey is a rich country in terms of plant genetic resources, and it is stated that there are near 12000 plant taxa (species, subspecies, variety) with the studies done in recent years (Avcı, 2005; Erik and Tarıkahya, 2004). However, more studies are needed to make a complete inventory of this richness of plant genetic resources and to bring these plants to the economy (Ekim *et al*., 2000).

Thyme is the most exported plant among medicinal and aromatic plants in Turkey (Ozguven *et al*., 2005). *Thymbra spicata* var. *spicata* L, (zahter) a species of thymbra, it grows naturally and intensively in Mediterranean countries and Turkey. However, the agricultural cultivation of the Zahter is not much done in Turkey and the plants collected mostly from nature and used.

Although Zahter collected from nature are mostly consumed in the region and in the domestic market, but their essential oil and spice are also exported. In addition to its traditional use as fresh in the region, the plants are dried and used as spice and tea, the essential oil of the plants is also used for different purposes. In particular, the use of fresh tip shoots is increasing day by day. The dried leaves of the plant are used as a spice in almost all dishes (soups, meat dishes, fish, herbed cottage cheese, pastries, etc.) for various purposes.

Like many other medicinal and aromatic plants, Zahter is used for various stomach aches and ailments diabetes and colds due to its antimicrobial and antiseptic properties (Hancı *et al*., 2003). In a study by Baydar *et al*. (2004) investigating the composition and antibacterial effects of essential oils of *Origanum*, *Thymbra* and *Satureja* species, it was determined that the most active antibacterial effect was the essential oil of the *Thymbra spicata* species.

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Saraç and Tunç (1995) determined that some essential oils have toxic and repellent effects, and that the essential oil of *Thymbra spicata*, has a repellent effect against the adults of the rice lice pest (*Sitophilus oryzae*). The importance and use of the Zahter is increasing day by day due to the increasing as use of the traditional food and the characteristics of its essential oil. As a matter of fact, many researchers have made researches on the cultivation of plants (Tonçer and Kızıl, 2005; İnan *et al*, 2011). The morphology, anatomy of zahter and the amount of essential oil content and components and antimicrobial properties of these compounds have also been studied by many researchers in Turkey (Doğan *et al*., 1987; Hancı *et al*., 2003; Özel *et al*., 2003; Baydar *et al*., 2004; Erken, 2005; Özcan *et al*., 2008).

There is not much research on the breeding of Zahter plant. In this study, it was aimed to create breeding lines by selecting single plant selection among plants grown naturally in different regions of Hatay province and to select high quality chemotypes and agrotypes with essential oil yield.

MATERIAL AND METHODS

Survey and collection studies of the Zahter (*Thymbra spicata* L.) plant were carried out. As a result of these studies, 213 single plants were selected and reproduced from 68 different locations and examined in terms of some characteristics. Healthy, different-looking and highly qualified individuals were selected in the pre-selection study. Since it is widely used for fresh consumption in the region, especially dense and large-leaved, highly branched types were also selected for this purpose during selection.

Clonal reproduction was achieved by taking cuttings from the selected plants. 1 part peat, 1 part soil and 1 part perlite were used as rooting medium in rooting tables. 1000 ppm IBA (Indole-3-butyric acid) was applied to the cuttings for 5 seconds. All of the rooted cuttings (213 plants) were planted at a distance of 50 x 70 cm in the collection garden and kept under protection.

The essential oil content of each plant was determined and essential oil components were also analyzed in GCMS and those with high carvacrol content were determined.

Leaf density: Each selected plant was evaluated in three groups as observation, sparse, middle and frequent.

Essential oil ratio (%): It was determined according to European pharmacopoeia with Neo-clavenger in 20 g sample taken from each plant. In the study, plant samples were distilled for three hours.

Essential oil components (%): It was determined by GC-MS in the essential oil samples obtained from each plant. Essential oil composition analysis was determined by using "Thermo Scientific ISQ Single Quadrupole" gas chromatography and mass spectrum in the Medical and Aromatic Plants Laboratory of Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops. A TR-5MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm x 30 m i.d, film thickness 0.25) column was used. Helium at 99.9% purity 1 mL / min was used as carrier gas. The ionization energy is 70 eV, the mass range is 1.2-1100 amu, the MS transfer line temperature is 250°C, the ion source temperature is 220°C, and the injection block temperature is 220°C. The samples were injected at a split ratio of 250. The injection amount was set as 1µl and the furnace temperature was adjusted to increase from 50°C to 220°C by 3°C / min. As a result of the analysis, each component was automatically identified by comparing the library mass spectra (Wiley and NIST) using Xcalibur software. Retention indices were

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calculated using standard n-alkane homologous solutions C8-C20 (Fluka, product code: 04070) and C21-C40 (Fluka, product code: 04071). Analysis of each sample on GC-MS takes 56 minutes.

RESEARCH AND DISCUSSION

Leaf Density

Each selected plant was evaluated in three groups as observation, sparse, middle and frequent and shown at Figure 1. It has been determined that frequent leaves common in general. Of the 213 plants, 138 of them have frequent leaves, 72 of them have medium density and 3 of them have sparse leaves. Leaf density is especially important in fresh consumption. In the locally consumed thyme salad, the ones with dense leaves are more preferred. For this reason, the density of leaves is important.

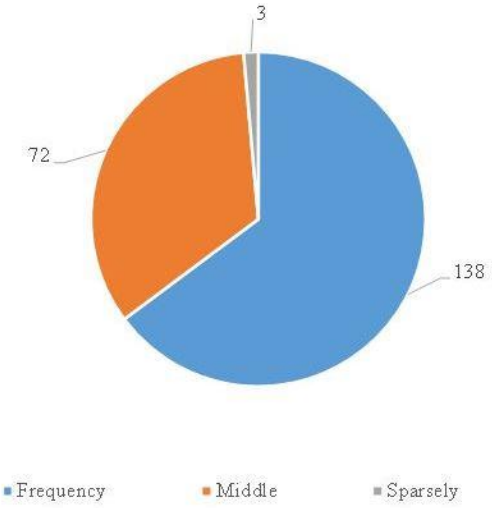

Figure 1. The distribution of leaf density of thyme genotypes

Essential Oil Ratio (%)

The essential oil ratios obtained as a result of the distillation of the leaves of 213 *Thymbra spicata* plants collected from the flora of Hatay were determined and given in Table 1. According to the results, the essential oil ratios of selected thyme plants varied between 0.70% and 3.90%. The values obtained have shown that the variation is greater by expanding the minimum and maximum values 1-3.4% given in the literature (Başer, 2002) in both directions. This situation reveals that the thymbra plants in the flora of Hatay show great differences in terms of essential oil ratios. The large variation requires the selection of types with a high rate of essential oils to be used in variety development. Among the collected thymbra plants, the varieties with code numbers Z14, Z3, Z25, Z38, Z77, Z104, Z35 and Z43, which are high in essential oil (over 3.50%), were selected to be used in future development studies.

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Table 1. The essential oil contents of leaves of collected *T. spicata* L. ecotypes

Code	Essential oil %	Code	Essential oil %	Code	Essential oil %	Code	Essential oil %	Code	Essential oil %
Z1	2,43	Z44	3,66	Z87	2,50	Z130	2,33	Z173	2,50
Z2	2,75	Z45	2,97	Z88	2,50	Z131	3,26	Z174	2,03
Z3	3,83	Z46	2,66	Z89	2,50	Z132	1,78	Z175	2,33
Z4	2,66	Z47	2,03	Z90	1,59	Z133	1,50	Z176	1,85
Z5	3,00	Z48	2,50	Z91	2,50	Z134	1,74	Z177	1,78
Z6	3,53	Z49	3,00	Z92	2,06	Z135	2,86	Z178	3,23
Z7	2,95	Z50	2,25	Z93	1,00	Z136	1,56	Z179	2,71
Z8	2,27	Z51	3,40	Z94	2,66	Z137	2,00	Z180	1,80
Z9	3,33	Z52	2,89	Z95	2,29	Z138	1,66	Z181	1,50
Z10	2,23	Z53	3,00	Z96	2,00	Z139	3,26	Z182	2,08
Z11	2,83	Z54	3,66	Z97	0,83	Z140	1,80	Z183	3,23
Z12	2,40	Z55	2,50	Z98	0,83	Z141	1,87	Z184	1,66
Z13	1,92	Z56	2,86	Z99	2,15	Z142	3,86	Z185	2,00
Z14	3,90	Z57	2,60	Z100	1,85	Z143	1,87	Z186	1,20
Z15	3,13	Z58	2,26	Z101	2,66	Z144	0,70	Z187	2,66
Z16	2,43	Z59	3,33	Z102	1,50	Z145	1,30	Z188	3,02
Z17	2,83	Z60	2,66	Z103	2,71	Z146	0,83	Z189	1,80
Z18	3,23	Z61	2,60	Z104	3,63	Z147	2,03	Z190	3,26
Z19	2,72	Z62	2,16	Z105	2,20	Z148	2,25	Z191	2,03
Z20	2,02	Z63	3,86	Z106	2,00	Z149	2,38	Z192	2,75
Z21	2,50	Z64	3,00	Z107	1,75	Z150	2,00	Z193	1,30
Z22	2,50	Z65	2,66	Z108	1,50	Z151	1,33	Z194	3,02
Z23	2,53	Z66	2,14	Z109	1,42	Z152	2,00	Z195	2,89
Z24	2,66	Z67	2,00	Z110	3,12	Z153	1,52	Z196	3,37
Z25	3,83	Z68	2,66	Z111	2,66	Z154	2,50	Z197	1,74
Z26	2,35	Z69	3,26	Z112	1,87	Z155	2,15	Z198	2,00
Z27	3,00	Z70	3,13	Z113	2,00	Z156	2,90	Z199	1,80
Z28	3,27	Z71	3,65	Z114	1,78	Z157	2,75	Z200	3,36
Z29	2,50	Z72	3,20	Z115	1,00	Z158	1,75	Z201	2,03
Z30	3,23	Z73	2,70	Z116	2,08	Z159	1,75	Z202	2,25
Z31	2,00	Z74	2,96	Z117	1,85	Z160	2,25	Z203	2,97
Z32	3,26	Z75	2,26	Z118	1,50	Z161	2,06	Z204	1,80
Z33	3,40	Z76	1,90	Z119	1,87	Z162	2,00	Z205	1,76
Z34	2,66	Z77	3,73	Z120	1,20	Z163	1,75	Z206	2,66
Z35	3,53	Z78	2,54	Z121	3,00	Z164	1,61	Z207	1,87
Z36	3,46	Z79	3,33	Z122	2,50	Z165	3,02	Z208	3,70
Z37	2,71	Z80	2,05	Z123	0,92	Z166	2,75	Z209	3,26
Z38	3,76	Z81	3,16	Z124	1,30	Z167	1,30	Z210	2,16
Z39	3,33	Z82	3,66	Z125	2,75	Z168	2,62	Z211	2,50
Z40	2,75	Z83	1,66	Z126	1,00	Z169	2,50	Z212	1,85
Z41	3,33	Z84	2,76	Z127	1,75	Z170	1,50	Z213	2,90
Z42	2,40	Z85	1,88	Z128	1,91	Z171	2,35		
Z43	3,50	Z86	3,00	Z129	3,33	Z172	1,60		

Min: 0.70; Max: 3.90; Average: 2.42; Std. deviation: 0.72; Coef. of variation: 29.53

Essential Oil Components (%)

The essential oil components obtained as a result of GC/MS analyzes in the study are given in Table 2, respectively (≥1%). The main component of thyme herbs is carvacrol. However, a wide variation has been detected among ecotypes in terms of carvacrol content. The carvacrol contents of the thyme plants varied between 28.12% (Z104, Z142) and 78.48% (Z167) and the average carvacrol ratio was 48.68% (Table 2).

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The prominent types in terms of carvacrol ratio were determined as Z167 (78.48%), Z165 (77.98%) and Z64 (72.54%).

Table 2. Carvacrol contents of the thymbra genotypes (%)

Code	Carvacrol %	Code	Carvacrol %	Code	Carvacrol %	Code	Carvacrol %	Code	Carvacrol %
Z1	55,66	Z44	56,25	Z87	49,00	Z130	30,32	Z173	67,08
Z2	49,95	Z45	51,83	Z88	59,77	Z131	44,49	Z174	49,02
Z3	54,37	Z46	42,87	Z89	39,54	Z132	50,28	Z175	39,13
Z4	54,50	Z47	59,88	Z90	40,08	Z133	40,40	Z176	48,05
Z5	59,25	Z48	45,52	Z91	38,51	Z134	35,63	Z177	47,76
Z6	48,61	Z49	56,38	Z92	45,07	Z135	47,58	Z178	56,63
Z7	50,52	Z50	47,87	Z93	49,61	Z136	41,89	Z179	39,32
Z8	55,66	Z51	49,11	Z94	49,17	Z137	43,45	Z180	40,92
Z9	52,41	Z52	50,41	Z95	41,63	Z138	42,94	Z181	57,80
Z10	54,49	Z53	54,49	Z96	52,33	Z139	41,63	Z182	37,82
Z11	47,81	Z54	51,24	Z97	32,71	Z140	46,44	Z183	47,84
Z12	49,65	Z55	53,67	Z98	51,62	Z141	47,00	Z184	53,77
Z13	59,66	Z56	32,20	Z99	66,77	Z142	28,12	Z185	56,64
Z14	49,80	Z57	47,83	Z100	46,83	Z143	39,48	Z186	60,23
Z15	51,05	Z58	49,02	Z101	49,01	Z144	52,44	Z187	44,74
Z16	46,30	Z59	47,32	Z102	33,52	Z145	34,92	Z188	44,28
Z17	58,18	Z60	44,37	Z103	56,25	Z146	43,10	Z189	51,60
Z18	56,03	Z61	61,76	Z104	28,12	Z147	41,13	Z190	39,63
Z19	51,02	Z62	50,90	Z105	56,58	Z148	43,74	Z191	38,39
Z20	56,18	Z63	52,02	Z106	28,84	Z149	56,96	Z192	42,80
Z21	56,39	Z64	72,54	Z107	40,07	Z150	30,32	Z193	59,10
Z22	48,17	Z65	47,50	Z108	66,57	Z151	42,96	Z194	54,05
Z23	31,28	Z66	52,35	Z109	55,63	Z152	49,23	Z195	64,09
Z24	59,64	Z67	73,66	Z110	48,85	Z153	43,79	Z196	36,36
Z25	44,74	Z68	48,85	Z111	36,39	Z154	54,41	Z197	45,79
Z26	59,16	Z69	53,94	Z112	37,11	Z155	37,68	Z198	50,53
Z27	53,49	Z70	51,39	Z113	52,16	Z156	59,96	Z199	38,13
Z28	54,84	Z71	52,52	Z114	39,10	Z157	41,89	Z200	31,37
Z29	45,60	Z72	53,95	Z115	64,85	Z158	41,52	Z201	39,59
Z30	49,00	Z73	58,74	Z116	38,28	Z159	44,53	Z202	48,31
Z31	54,74	Z74	32,94	Z117	53,94	Z160	38,72	Z203	45,66
Z32	47,92	Z75	61,76	Z118	58,74	Z161	46,16	Z204	51,42
Z33	57,00	Z76	45,76	Z119	32,87	Z162	41,40	Z205	48,93
Z34	41,02	Z77	58,74	Z120	37,70	Z163	47,00	Z206	49,33
Z35	46,30	Z78	29,79	Z121	44,35	Z164	69,89	Z207	46,13
Z36	53,15	Z79	32,87	Z122	40,05	Z165	77,98	Z208	51,23
Z37	44,26	Z80	49,91	Z123	50,02	Z166	38,83	Z209	50,28
Z38	44,05	Z81	47,98	Z124	54,83	Z167	78,48	Z210	70,53
Z39	49,01	Z82	45,87	Z125	41,21	Z168	68,29	Z211	34,60
Z40	47,94	Z83	47,58	Z126	41,64	Z169	57,38	Z212	48,26
Z41	51,28	Z84	46,12	Z127	44,23	Z170	63,00	Z213	51,15
Z42	59,64	Z85	52,10	Z128	43,56	Z171	54,37		
Z43	49,35	Z86	44,15	Z129	47,98	Z172	51,39		

Min: 28.12; Max: 78.48; Average: 48.68; Std. deviation: 9.19; Coef. of variation: 18,89

Generally, p-Cymene is seen as the second component. Even in some ecotypes, p-Cymene values were obtained almost similar to carvacrol. In addition, some ecotypes contain thymol with carvacrol. Z23 (18.18%), Z41 (16.88%), Z56 (20.75%), Z74 (21.51%), Z79 (7.41%), Z119 (8.56%), Z130 (16.00%) and Z150 (18.21%) code numbered ecotypes contain thymol at specified rates. The results obtained are in agreement with the literature (Başer *et al*., 1996, Hancı *et al*., 2003).

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The zahtar that were collected were examined in terms of leaf density, essential oil ratio and essential oil components. Among the zahtar collected in the study, the plants with code number Z14, Z3, Z25, Z38, Z77, Z104, Z35 and Z43, which are high in essential oil (over 3.50%), were selected. The prominent types in terms of carvacrol ratio were determined and selected as Z167 (78.48%), Z165 (77.98%) and Z64 (72.54%). In the study, it was also determined that some plants with low essential oil content such as Z144 and Z158 also have low oil glands number and oil glands diameter. It would be appropriate to evaluate these values obtained as preliminary knowledge together with future ontogenetic variability studies.

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