



Characterization of *Origanum vulgare* SUBSP. *hirtum* (LINK) Iestwaart Population and Determination of A Clones

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Abstract

Identification of the high yielding and the good quality clones of one population (B population) of *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart was one of the purposes in this study and furthermore some agronomic and quality characteristics of individual plants were examined during two years (2014-2015). Annual performances were determined according to the minimum and maximum values of the first, second and non-flowering harvests of the population. Also for second year, minimum, maximum and mean values of the individual plants of the population were stated in the paper. The aim was to reveal if the high yield and quality characteristics of plants are based on the genotype. Individual plants of the B population, the mean plant height was found as 39 cm, canopy value as 30.5 cm, Fresh herb yield as 167.9 g/plant, Drog herb yield as 65.5 g/plant, Drog leaves yield as 32.2 g/plant and the mean rate of the essential oil as 4,01%. Considering the two years' results of the B population, A clones were created in 2016 by selecting the high yielding and of good quality genotypes. On A clones, the plant height, fresh herb yield, Drog herb yield, essential oil rate and essential oil yield values were determined between 13-52.5 cm, 8-624 g/plot, 4-218.4 g/plot, 1.6-100.1 g/plot, 1.08-7.92 % and 0.03-5.74 L/parcel, respectively.

Key Words: *Origanum vulgare* subsp. *hirtum*, Population, Selection, Yield, Essential Oil, Compound

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1. Introduction

The importance of the medicinal plants and herbs increases day by day. In Turkey, a great majority of these plants are being picked up from the nature; consequently, environmental destruction and the loss of some endemic species are inevitable. The primary of these plants is oregano which is being picked from the flora and has a crucial role in export. In Turkey, the genus involving different types of oregano are *Origanum*,

Thymbra, *Thymus*, *Coridothymus* and *Satureja*. The species which are exported most and used in the production of essential oils are *Origanum onites*, *Origanum vulgare* subsp. *hirtum*, *Origanum minutiflorum*, *Origanum majorana* and *Origanum syriacum* var. *bevanii*. Beside these, other traded species are *Coridothymus capitatus*, *Thymbra spicata*, *Thymbra sintenissii*, *Satureja cuneifolia*, *Satureja hortensis*, *Satureja montana*, *Satureja spicigera* and *Thymus eigii*. The common quality of all these species

is containing essential oils and the main components of their essential oils are Carvacrol or Thymol (Başer, 2001; Hayta and Arabacı, 2011). Recently, Lukas et al. (2013) indicated that *Origanum dubium* and *Origanum majorana* should be better classified as different species even though they are morphologically similar. Marjoram, which has been known as *O. majorana* so far, actually belongs to the species *O. dubium*. In Turkey, the most valuable oregano species belong to the genus *Origanum* in economical and agricultural fields.

In Turkey, approximately 80% of the oregano which is still exported is cultivated under field conditions, 20% is picked up from the nature (Bayram et al. 2010). Oregano picked up from the nature belong to the various genuses and species such as *Coridothymus capitatus*, *Thymbra spicata*, *Origanum onites*, *Origanum syriacum*, *Origanum majorana*, *Origanum minutiflorum* and *Origanum vulgare* subsp. *hirtum* (TSI, 2004). Cultivated oregano species are *Origanum onites* and *Origanum vulgare* subsp. *hirtum* which are mainly grown in Marmara, Mediterranean and Aegean Regions.

This study is done with the purpose of the characterization of selected promising plants at the B clone of *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart and the development of new types with higher Drog yields and essential oil rates using the clone selection method.

2. Material and Method

The seeds of B population is used in study that is one of the five promising populations (A,B,C,D and E). All populations were selected from the *Origanum vulgare* subsp. *hirtum* (Link) Iestwaart plants. The study carried out in Aydın ecological conditions. The B population is originated from the south east exposure of the Ida Mountains with the altitude of 559 m and the mapping coordinates of 545202-4423405.

The field study of the experiment was carried out on the area of the Research and Practice Farm of the Adnan Menderes University, Faculty of Agriculture. The individual plants were investigated during the years of 2013-2014 and 2014-2015 and A clones were cultivated during the year 2016. Study field has typical Mediterranean climate conditions, the total precipitation of long years is 636,7 mm and the average temperature is 17,7°C. The soil texture of the study area was sandy-loam, pH value is 8.43 and the ratio of the organic substances were 1.30%.

The seeds of the B population were sown in 06/12/2013 on the seedbeds in a greenhouse and the germination was observed in 17/12/2013. When plants have proper maturity (8-10 cm seedlings), to create the individual plants, 648 seedlings were planted to an area of 129.6 m² with a planting level of 50x40 cm in 02/04/2014. The flowering individual plants of the population were harvested in 10-18/07/2014 and in 10/09/2014 during the first year. Some plants were harvested once and some of them were harvested twice. While the plants were harvesting (10-18/07/2014 and 10/09/2014), some of the individual plants of population B haven't flowered so the harvesting could not be performed on these plants. To determine the annual growth and yield performance of the individual plants for the first year, just after identifying the morphological characteristics of the plants without taking the flowering or nonflowering into account they were harvested between the dates of 14-20/10/2014 and 04-07/11/2014. During the harvest between these dates even some plants have reached to the maturity for a third harvest. In order to determine the individual plants those have good quality features, observations and measurements were repeated for two years. The harvestings of the second year were done on the dates 24/06/2015 and 07/07/2015.

The analyses of the essential oil were performed in the Medicinal Plants Laboratories of Adnan Menderes University and Ege University Agriculture Faculties. The essential oil rates of the study were determined volumetrically on the air dried leaves samples using the Neo Clevenger apparatus. The essential oil rates are stated as milliliter /100 g (%) on air dried leaves (Wichtl, 1971).

Among the plants on the field, the promising individual ones were identified from B populations to form A clones considering the results of the yield of leaves and essential oil of two years and The superior 140 genotypes were selected.

From 140 selected genotypes, for each genotype the 10 cuttings planted in the greenhouse between 03-05/12/2015. Nevertheless, upon determining that four genotypes are not suitable for the vegetative reproduction so final number of the genotypes planted on the field was 136. After rooting of cuttings, between dates of 06/04/2016 and 07/04/2016, the A clone cuttings were planted to the 2 m² plots with a planting density of 50 x 40 cm and 10 plants belonging to the each row. So the A clones were created. On the third year of the trial, all the crop care was performed properly on the A clones. The observations and measurements were done firstly and after that the first and last plants of each clone were left as the border effect and the remaining plants were harvested on 27/06/2016.

The essential oil components were determined with GC-MS (Shimadzu 2010 Plus QP-5050 Quadrapole Detector) at the SDU Experimental and Observational Research and Application Center. GC-MS operating conditions were carried out as follows: CP-Wax 52 CB (50 m x 0.32 mm, 0.25 µm) capillary column was used, column temperature was initially 60°C, then gradually increased to 220°C at 10°C/min

and waited for 10 minutes at 220 °C. The total analysis time was 60 minutes, the injector temperature was 240°C and the detector temperature was 250°C. Helium (20 mL/min, split 1:20) gas was used as the carrier gas. 7.5 µl of the sample was diluted by adding 1500 µl of dichloromethane. Wiley, Nist, Tutor, FFNSC libraries were used to identify the essential oil components.

The findings were evaluated statistically using the SPSS (SPSS 17.0 2008) and TOTEMSTAT (Acikgoz et al., 2004) programs.

3. Results

The statistical findings of the two years (2013-2015 growth years) which belong to the individual plants of the B population of *O. vulgare* subsp. *hirtum* (Link) Iestwaart grown under Aydın ecological conditions and A clones created from these population were stated below;

3.1. Individual plants

Within 648 individual plants, 581 plants were reached maturity for harvest. The first year of the study, flowering was viewed on the 208 plants of the B population and the harvesting was done for those individual plants. Some statistical analyses of the characteristics belonging to the first harvest of the B population are shown on Table 1. According to the mean values, plant height, canopy value, Fresh herb yield, drog herb yield, drog leaves yield and essential oil rate have been determined as 35.3, 17.8, 56 g/plant, 21 g/plant, 14.6 g/plant and 4.15%, respectively. It was observed that after the first harvest, some of the individual plants of the B population kept growing and some plants have had second flowering so the second harvest were made with those 11 flowering individual plants. The plant height of the harvested plants was 28-67 cm, canopy value 20-36, fresh herb yield 8-83 g/plant, drog leaves yield 3.95- 47.58 g/plant and the essential oil rate varied between 3-4.7% (Table 2).

Table 1. Statistical values for the first year (2014) I. harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	208	14.000	50.000	35.327	50.7235	7.1220	0.4938	20.1604
Canopy Value (cm)	208	3.000	45.000	17.827	51.6704	7.1882	0.4984	40.3222
Fresh Herb Yield (g/plant)	208	12.000	185.000	55.966	776.5931	27.8674	1.9323	49.7932
Drog Herb Yield (g/plant)	208	2.000	90.000	21.000	144.7826	12.0326	0.8343	57.2979
Drog Leaves Yield (g/plant)	208	1.110	42.240	14.636	58.5139	7.6494	0.5304	52.2653
Essential Oil Rate (%)	208	1.100	7.300	4.147	1.2583	1.1217	0.0778	27.0502

Table 2. Statistical values for the first year (2014) II. harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	11	28.000	67.000	44.273	178.4182	13.3573	4.0274	30.1706
Canopy Value (cm)	11	20.000	36.000	29.182	38.5636	6.2100	1.8724	21.2802
Fresh Herb Yield (g/plant)	11	17.000	200.000	97.091	4961.0909	70.4350	21.2370	72.5454
Drog Herb Yield (g/plant)	11	8.000	83.000	40.455	859.0727	29.3099	8.8373	72.4515
Drog Leaves Yield (g/plant)	11	3.950	47.580	24.239	311.8603	17.6596	5.3246	72.8557
Essential Oil Rate (%)	11	3.000	4.700	3.700	0.3000	0.5477	0.1651	14.8033

When table 3 was examined, it was seen that 530 individual non-flowering plants were harvested in the first year. On this harvest, plant height varied between 12-67 cm and the mean value has been 32.4 cm. The minimum and maximum canopy values were 20 cm and 73 cm, respectively and the mean canopy value was found as 44.7 cm. The yield of the fresh herb varied between 20-699 g/plant and the mean yield was 229.85.

Minimum drog herb yield was 4 g/plant, maximum herb yield was 262 g/plant and the mean yield has been 88.75 g/plant whereas yield of drog leaves varied between 3.3-119.79 g/plant and the mean leaves yield has been found as 41.13 g/plant. Minimum and maximum essential oil rates of the plants were 0.2% and 2.5%, respectively and the mean value was stated as 1.26%.

Table 3. Statistical values for the first year (2014) non flowering harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	530	12.000	67.000	32.389	93.7050	9.6801	0.4205	29.8874
Canopy Value (cm)	530	20.000	73.000	44.740	78.4992	8.8600	0.3849	19.8034
Fresh Herb Yield (g/plant)	530	20.000	699.000	229.847	11591.6609	107.6646	4.6767	46.8418
Drog Herb Yield (g/plant)	530	4.000	262.000	88.745	1679.1430	40.9773	1.7799	46.1740
Drog Leaves Yield (g/plant)	530	3.300	119.790	41.132	395.9682	19.8989	0.8644	48.3780
Essential Oil Rate (%)	530	0.200	2.500	1.264	0.1375	0.3708	0.0161	29.3280

Table 4. Statistical values for the second year (2015) harvest of the individual plants of B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	581	9.000	85.000	40.738	219.2107	14.8058	0.6142	36.3435
Canopy Value (cm)	581	7.000	55.000	30.306	94.7715	9.7351	0.4039	32.1222
Fresh Herb Yield (g/plant)	581	7.000	925.000	208.098	25474.2093	159.6064	6.6216	76.6977
Drog Herb Yield (g/plant)	581	3.000	407.000	80.972	4027.9406	63.4661	2.6330	78.3798
Drog Leaves Yield (g/plant)	581	1.620	170.700	37.791	751.8826	27.4205	1.1376	72.5580
Essential Oil Rate (%)	581	1.800	8.500	4.991	0.9451	0.9722	0.0403	19.4777

On the second year of the study, 581 individual plants were harvested. Some statistical values of the second year are shown on table 4. The plant height varied between 9-85 cm and the mean value was determined as 40.7 cm. The mean canopy value was 30.3 cm, minimum and maximum values have been 7 and 55 cm, respectively.

The mean values of fresh herb yield, drog herb yield and drog leaves yield has been 208.1 g/plant, 80.97 g/plant and 37.79 g/plant, respectively. The minimum essential oil rate was 1.8 % whereas the maximum value was 8.5 % and the mean value was 4.99% (Table 4).

Table 5. Mean harvest values (2014-2015) of the individual plants of B population *Origanum vulgare* subsp. *hirtum* for two years

Characteristics	1.Year I. Harvest	1.Year II. Harvest	1.Year Non Flowering Harvest	1.Year Mean Harvest Values	2.Year Harvest	Mean Values for Two Years
Plant Height (cm)	35.327	44.273	32.389	37.330	40.738	39.034
Canopy Value (cm)	17.827	29.182	44.740	30.583	30.306	30.445
Fresh Herb Yield (g/plant)	55.966	97.091	229.847	127.635	208.098	167.867
Drog Herb Yield (g/plant)	21.000	40.455	88.745	50.067	80.972	65.520
Drog Leaves Yield (g/plant)	14.636	24.239	41.132	26.669	37.791	32.230
Essential Oil Rate (%)	4.147	3.700	1.264	3.037	4.991	4.014

The mean values of the two years of the examined characteristics of B population were shown on table 5. The plant height was found as 39 cm, canopy value as 30.4 cm,

fresh herb yield as 167.9 g/plant, drog herb yield as 65.5 g/plant, drog leaves yield as 32.2 g/plant and the mean rate of the essential oil has been 4%.

Table 6. Statistical values of some characteristics of A clone belonging to the B population *Origanum vulgare* subsp. *hirtum*

Characteristics	Number of Plants	Minimum	Maximum	Mean	Variance	Standard deviation	$S^{\bar{x}}$	CV
Plant Height (cm)	136	13.000	52.500	33.567	85.233	9.232	0.792	27.504
Fresh Herb Yield (g/plant)	136	8.000	624.000	186.965	22655.392	150.517	12.907	80.506
Drog Herb Yield (g/plant)	136	4.000	218.400	68.660	2706.207	52.021	4.461	75.767
Drog Leaves Yield (g/plant)	136	1.600	100.100	31.689	423.865	20.588	1.765	64.968
Rate of Leaves-Stem (%)	136	20.800	80.000	52.056	183.635	13.551	1.162	26.032
Essential Oil Rate (%)	136	1.080	7.920	4.529	1.674	1.294	0.111	28.568
Essential Oil Yield (g/plot)	136	0.030	5.740	1.534	1.286	1.134	0.097	73.942

Table 7. Essential oil Components of A clones in B Population (%)

Component Names and Ratios (%)										
Clone No	TCC * (Quantity)	α -Pinene	2- β -Pinene	β -Myrcene	α -Terpinene	ρ -Cymene	γ -Terpinene	Thymol	Carvacrol	Essential Oil Ratio (%)
B-4	11	0.85	1.53	-	-	3.55	14.44	0.04	76.91	6.83
B-20	12	1.45	2.14	-	1.54	3.68	11.32	0.16	76.64	6.25
B-70	16	0.37	1.06	-	0.74	2.93	6.36	0.12	84.45	5.50
B-102	13	-	1.64	-	1.32	3.47	13.59	0.28	76.52	6.92
B-183	18	0.62	0.04	1.18	0.89	2.80	5.99	0.07	83.48	5.75
B-201	15	1.29	-	1.55	0.70	3.42	3.85	0.17	84.95	5.75
B-296	14	1.16	-	1.43	0.87	3.26	3.72	0.16	86.02	5.50
B-342	11	2.80	-	2.53	2.72	8.54	16.67	0.71	62.17	5.75
B-345	15	1.39	2.09	-	-	3.87	13.25	0.02	75.97	6.00
B-368	14	0.93	-	1.43	1.21	3.30	9.67	0.11	80.89	7.25
B-413**	10	0.19	-	-	2.72	1.51	0.13	-	92.14	5.13
B-414	12	0.77	1.14	-	0.80	3.24	5.21	0.03	86.01	5.42
B-417	12	0.60	1.35	-	-	3.31	6.70	0.08	83.90	6.33
B-423	13	0.84	1.10	-	0.83	3.08	4.41	0.05	87.00	5.46
B-430	15	0.60	-	1.12	1.11	2.87	8.77	0.03	83.12	5.58
B-446	50	0.47	-	1.14	0.56	2.54	2.73	0.03	86.92	6.33
B-447	16	0.49	1.20	-	0.55	1.92	3.08	0.11	88.50	6.67
B-458	12	0.41	-	0.78	-	2.68	3.11	0.16	89.92	5.25
B-466	8	3.27	3.66	-	-	-	21.51	0.21	69.99	5.92
B-575	15	1.45	2.08	-	-	4.68	13.26	0.04	74.06	5.33

* Total Components Contents = TCC

** The B-413 clone also contains 2.05% Alloaromadendrene.

3.2. A clones

The findings of the variance, standard deviation, mean values and coefficient variation of the A clone were shown on table 6. According to the results of the statistical analyses, the minimum and maximum values have been determined for plant height 13-52.5, for fresh herb yield 8-624 g/plot, for drog herb yield 4-218.4 g/plot, for drog leaves yield 1.60-100.1 g/plot, for the ratio of leaf-stem 20.8-80%, for the essential oil rate 1.08-7.92% and for the essential oil yield 0.03-5.74 g/plot. On the other hand, coefficient variation has been found significantly high for the characteristics of fresh herb yield, drog herb yield, drog leaves yield and essential oil yield.

From each A clones, 20 clones have the maximum values or higher values than the

average for the characteristics of fresh herb yield, drog herb yield, drog leaves yield and essential oil rate were selected for the further research.

4. Discussion

According to the years, some growth variations are observed on the individual plants. Second year of plant development, root system develop better and vegetation period is longer so an increased yield could be expected.

A wide variation was identified among the examined B population. A similar situation was stated on the studies of Ceylan et al. (2003) examined yield potential and essential oil composition of *Origanum onites* L. clones and individual plants and Arabacı et al. (2012) who studied on the ontogenetic variability of *Coridothymus capitatus* L. genotypes. Our results are in accordance with

the findings of the researchers. While the plant height values of the study are similar to the values of Žukauska (2001) as 47-53 cm from nature, 32-93 cm cultivated, Oflaz et al. (2002) as 75-80 cm, W'glarz et al. (2006) as 54-68 cm and Ahmad et al. (2008) as 25.45-55.58 cm. Our maximum plant height values are close to the cultivated plant heights of Žukauska (2001).

Žukauska (2001) obtained a fresh herb yield of 82-915 g/m² from the natural area of Latvia, fresh herb yields of cultivated population of this plants have been for the first, second and third years 113-1050 g/m², 125-2135 g/m² and 516-2414 g/m², respectively. Our fresh herb yields have similarity to the results of the researcher.

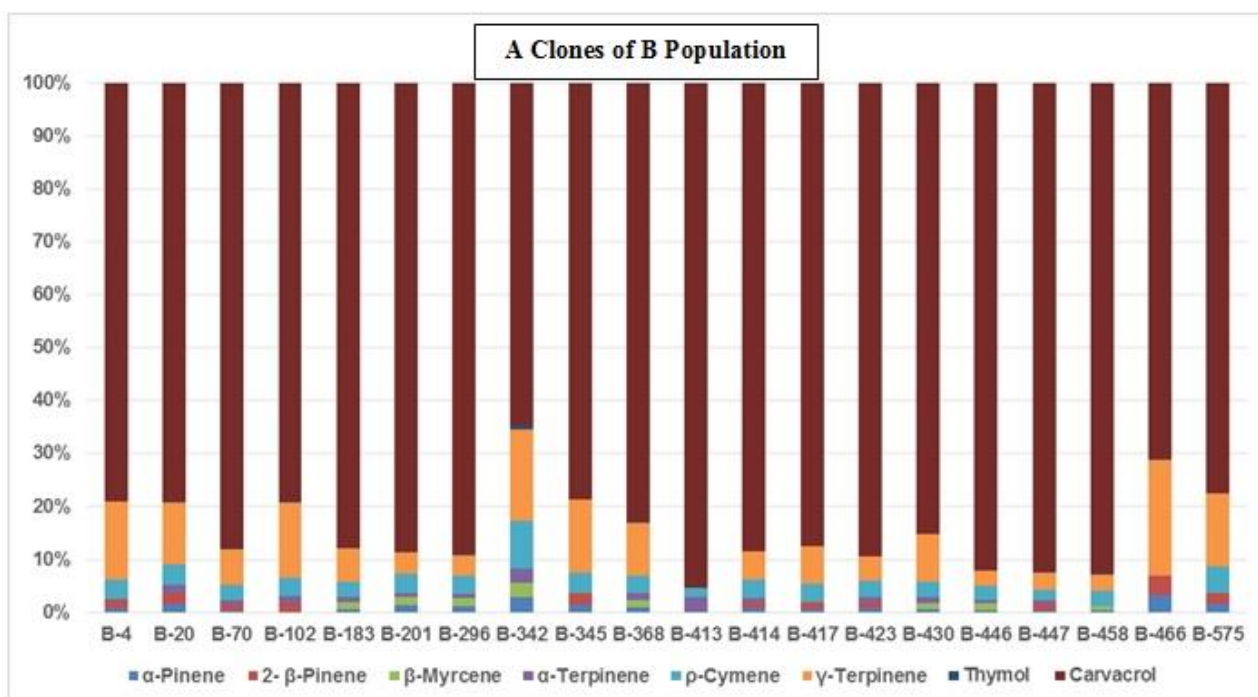


Figure 1. Distribution of Essential Oil Components of A Clones for Population B (%)

Leto and Salamone (1996) reported that the drog herb yield of the natural population varies between 105-1100 g/plant. W'glarz et al. (2006) stated that the values for this characteristic are between 243.1-636.6 g for a single plant. While the findings we obtained from our research are in the range of the findings of W'glarz et al. (2006), they have low values compared to the maximum values of Leto and Salamone (1996).

Kokkini (1996) reported that the essential oil yield of *O. vulgare* subsp. *hirtum* samples from different locations were between 1-1.6 ml/100g. Tinmaz et al. (2002) determined the quality characteristics of *O. vulgare* subsp. *hirtum* from various cities of Marmara Region

and they observed that the essential oil rate increases when the plants are transferred to a cultivated area from nature. Study also has mentioned that the maximum essential oil yield was 12.3 kg/da. Said-Al Ahl et al. (2009) indicated that the essential oil rate is between 0.070-0.072 ml/plant. Our findings are in accordance with the results of Said-Al Ahl et al. (2009).

Tinmaz et al. (2002) identified that the essential oil rate varies between 1-6.1% on the *O. vulgare* subsp. *hirtum* samples picked up from the nature, whereas the natural plant samples of Leto and Salomone (1996) include 4% essential oil. Başer (2001) found a range for the essential oil rate between 1-7% and

Oflaz et al. (2002) found 3.6-4.4 %. They reported a value of 3.9% for a plant sample from Kaz Mountains. Veres et al. (2003) specified the essential oil rate of *O. vulgare*

subsp. *hirtum* as 4.3 %. Oil rate of an individual plant was reported between 1.1-3.2% by W'glarz et al. (2006).

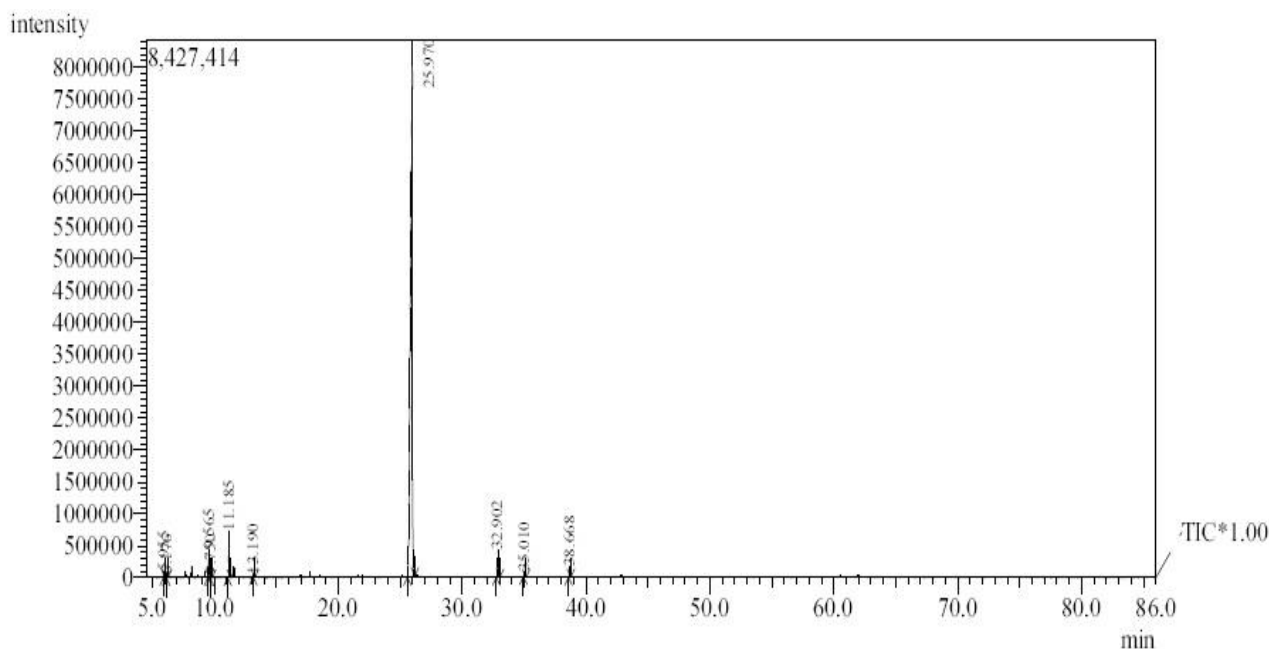


Figure 2. GC / MS Chromatogram of B-413 Clone

Sancaktaroğlu and Bayram (2011) determined the essential oil rate of cultivated plants between 3.78-4.59%. Karamanos and Sotiropoulou (2013) observed an increase of essential oil rates which were 1.5% on the second season and 2% on the third. Lukas et al. (2015) examined the essential oil content of 502 *Origanum vulgare* individual plants from 17 different countries and 51 populations and reported that the values varies between 0.03-4.6%. Our findings are considerably higher than the results of the other researchers.

5. Conclusions

When all the results are generally evaluated, there was found wide genotypic variation among the examined population. For first year of the study, the values of agronomical features of first harvest were lower than the second and the non-flowering (third) harvest. However maximum essential oil rate values were obtained from the first harvest.

For second year of the study, there were increase on values of plant height, canopy, fresh herb yield, drog herb yield, drog leaves yield and essential oil rate. Differences among genotypes in terms of yield and quality were determined in this study. The population was evaluated in the sense of agronomy and quality and a major variation was observed. According to the results, genotypes with higher yield and essential oil rates have been selected and A clones were created. Among A clones, 20 superior clones were selected for the further studies. It could be possible in the future to complete the breeding circle of clone selection with further studies.

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Conflict of Interest

The authors have declared that they have no conflict of interest.

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