



Identifying superior genotypes by clonal selection in Istanbul Oregano (*Origanum vulgare* subsp. *hirtum* (Link) A.Terracc.) populations

Olca Arabacı · Emine Bayram · Uğur Tan · Çiğdem Sönmez

Received: 25 November 2024 / Accepted: 2 May 2025 / Published online: 21 May 2025
© The Author(s) 2025

Abstract Oregano has been valued for its medicinal and aromatic qualities since ancient times and continues to be extensively used today. Istanbul oregano (*Origanum vulgare* ssp. *hirtum*) is particularly important and distinguished from other subspecies by its high essential oil content. This study aimed to identify high-yielding and high-quality genotypes from selected populations which are important for its high essential oil content, and to develop superior varieties through clonal selection. The study was conducted over two years at the experimental field and agronomic and quality traits of five different populations (A, B, C, D, and E), all from the carvacrol chemotype, with a total of 3324 genotypes were evaluated. The minimum, maximum, and average values for plants from each population were determined. The two-year averages showed that plant height ranged from 34.5 to 41.8 cm, fresh herb yield from 78.8 to 176.6 g/plant, dry herb yield from 31.9 to 70.1 g/plant, and dry leaf yield from 17.0 to 36.9 g/plant. Also, it was observed that the minimum and maximum essential oil contents of individual plants were higher in the second year compared to the first. The essential oil content

for the second year varied across the different populations: Population A ranged from 0.8 to 9.0%, Population B from 1.8 to 8.5%, Population C from 1.0 to 10.0%, Population D from 1.1 to 8.7%, and Population E from 1.0 to 9.8%. Based on these results, genotypes with both high yield and significant essential oil content were selected in the end of study for further improvement through clonal selection.

Keywords Breeding · Population · Selection · Single plant · Yield · Essential oil

Introduction

Oregano has been valued for its medicinal and aromatic qualities since ancient times and continues to be extensively utilized today. It plays a crucial role in the global spice market and is integrated into diverse industries, ranging from medicine and pharmacy to cosmetics, essential oils, food production, and even landscaping and organic farming (Arabacı 2022). The Mediterranean region, particularly the eastern part, is the primary source of global oregano production, accounting for approximately 75% of the species distribution (Başer et al. 1993). The *Origanum* genus includes many species, with *Origanum vulgare* being notably diverse. Four distinct subspecies of *Origanum vulgare* (subsp. *gracile*, subsp. *hirtum*, subsp. *viride*, and subsp. *vulgare*) are found in Türkiye alone (Baytop 1999). Approximately 60% of the known

O. Arabacı · U. Tan (✉)
Field Crops Department, Faculty of Agriculture, Aydın
Adnan Menderes University, 09100 Aydın, Turkey
e-mail: ugur.tan@adu.edu.tr

E. Bayram · Ç. Sönmez
Field Crops Department, Faculty of Agriculture, Ege
University, 35100 İzmir, Turkey

Origanum species in the world are found in Türkiye, providing strong evidence that the country serves as a genetic center for *Origanum* species (Başer 2001). *Origanum vulgare* subsp. *hirtum* is a commercially valued species exported from Türkiye and it is commonly known as Istanbul oregano or Çanakkale oregano (Ofiaz et al. 2002). It is particularly important and distinguished from other species by its high essential oil content, as its essential oil yield is greater compared to other oregano subspecies (Esen et al. 2007).

In plant breeding, clone selection is a technique used to find genetic variants within a population and utilize them to create plants with desired characteristics. This technique helps to improve agricultural productivity, by identifying individual plants within populations that show favorable characteristics, such as high yield or specific quality traits. It is especially useful in vegetatively propagated crops, such as fruit trees, potatoes, and ornamental plants, where it is important to maintain plant's genetic structure. The process involves careful evaluation and selection of superior clones within populations, and then multiplying to produce genetically identical offspring. This ensures that advantageous traits are retained across generations (Tuğlu and Baydar 2019). Medicinal plants are often collected from the wild, cross-pollinated species offer significant genetic variation for selecting high-yielding genotypes in populations (Nasrallah and Nasrallah 1993; Ferrer et al. 2009). The primary selection criteria for these plants are essential oil content, pharmacologically active compounds, and desirable agronomic traits. The Yenice region, particularly in the Kazdağları area with its Mediterranean climate, consists of extensive mountainous regions that are largely inaccessible to people, making it a valuable area in terms of genetic diversity. Due to climate change, preserving genetic resources through cultivation has become crucial. Cultivating these plants, rather than overharvesting, helps maintain biodiversity while producing commercially valuable products that meet market standards. Developing new varieties through appropriate breeding methods is key to enhancing their economic value and ensuring sustainable use, ultimately protecting both the ecological and commercial future of medicinal plants.

The aim of this study was to develop new varieties through clonal selection in Istanbul oregano (*Origanum vulgare* subsp. *hirtum*), which has gained attention in recent years for its high essential oil content.

By evaluating selected populations for their high yield and quality traits, the study seeks to identify superior genotypes suitable for breeding purposes.

Material and methods

Plant material

Populations of *Origanum vulgare* subsp. *hirtum* are spread naturally over diverse altitudes and geographic locations in the Kazdağları Yenice area of Türkiye. These populations grow naturally in dry hills, rocky ridges, forest openings, slopes, mixed forests, and partially shaded areas (Baytop 1999; Birinci 2008). It is a perennial plant that can grow up to 60 cm tall, has stems that are generally covered with soft hairs, densely gland-dotted leaves, and dense, compact flowers. The branches and spikelets are woody. The plant bears white or pink flowers, and has a strong aroma, with a tubular calyx with five lobes, and a corolla 3–10 mm long with stamens extending slightly beyond the corolla (Fig. 1.), (Davis 1982; Baytop 1999; Kıtık et al. 1997). The plant is cross-pollinated and can be propagated both vegetatively and by seed.

Method

A total of 165 populations of *Origanum vulgare* subsp. *hirtum* were identified across the Yenice region of Türkiye, and plant samples were systematically collected from each of these populations. Essential oil



Fig. 1 *Origanum vulgare* subsp. *hirtum* (Link) A.Terracc.), **a** vegetative stems with leaves, **b** inflorescences with flowers

analyses were performed on the collected samples to determine their essential oil content. Populations with high essential oil content were selected (41 populations) for further study, and seeds of these populations were collected for propagation (seedlings) under greenhouse conditions. Field trials were established using seedlings from 41 populations; however, 2 populations showed poor adaptation, so study continued with 39 populations. Up to this point, the study was conducted as part of the TÜBİTAK 1040601 project for 5 years (Fig. 2). In this study, agronomic and quality traits obtained from these 39 populations were evaluated, and based on these traits, five promising populations, all from the carvacrol chemotype, were selected and labeled with letters (A, B, C, D, and E) (Table 1, Fig. 3). These five populations were evaluated for 2 years (2014 and 2015) in the field conditions of the Department of Field Crops, Faculty of Agriculture, Aydın Adnan Menderes University, in Aydın Province. Oregano is an open-pollinated plant capable of propagation both by seed and vegetative means. Therefore, a clonal selection method was employed in this study (Demir and Turgut 1999).

Field experiment location

Experiment field (37°45'43" N 27°45'31" E, elevation 28.2 m) is in Aydın province, it is located in the Mediterranean Flora region and is influenced by a temperate Mediterranean climate. According to long-term averages for the region where the research was conducted, total annual precipitation is recorded as 636.7 mm, and the average temperature is 17.7 °C. The average temperatures were 17.6 °C in 2014, and 16.9 °C in 2015. The total precipitation was measured 754.4 mm in 2014, and 673.2 mm in 2015, while relative humidity was 73.9% in 2014, and 72.0% in 2015 (Fig. 4).

The soil tests of the experimental field were found to be sandy-loam in texture, calcareous (3.80%), alkaline (pH 8.43), and low in organic matter (1.30%). In terms of nutrient content, the soil had a moderate level of phosphorus (17 ppm), low potassium (182 ppm), and high levels of calcium (3560 ppm), magnesium (301 ppm), and iron (23.0 ppm). Zinc content was at a critical level (0.70 ppm), while manganese (8.0 ppm), copper (2.0 ppm), and boron (1.21 ppm) were found to be sufficient. The sodium content of the soil was determined to be very low.

Experimental design

To develop a cultivar of *Origanum vulgare* subsp. *hirtum* using the clone selection method (Hoffman et al. 1971; Demir and Turgut 1999) to ensure the traceability of the selected genotypes in the future, each population collected from different locations was planted separately in the field. The seedlings were transplanted into the field at a spacing of 50×40 cm, with each population designated to its specific plot. The plots had the following areas: 140.4 m² for population A, 129.6 m² for population B, 140.4 m² for population C, 129.6 m² for population D, and 129.6 m² for population E, making up a total experimental area of 669.6 m². A total of 3324 seedlings were transplanted across these plots, with 702 seedlings from population A, 648 from population B, 702 from population C, 636 from population D, and 636 from population E. Irrigation was carried out using the furrow method when needed.

Harvesting procedure

Harvesting of individual plants from the five populations was carried out during the full flowering period on 10–18th July and 10th September. Some plants were harvested for the first time, while others were harvested for the second time. During harvesting, plants from each population were individually collected, placed in paper bags, and weighed to determine their fresh weight. For dry yield determination, plant samples were placed on drying racks and left under room conditions (20–28 °C). After approximately 4–5 days, the dried samples were weighed to determine the dry herb yield, and after removing the larger stems, the dry leaf yield (containing leaves, inflorescences and thin stems) was also recorded.

During the harvesting periods (10–18 July and 10 September), some individual plants from the populations (A, B, C, D, and E) did not flower, and therefore, harvesting could not be performed. In order to determine the annual growth and yield performance of individual plants in the first year, all plants were harvested between 14 and 20 October and 04–07 November, immediately after their morphological characteristics were recorded, regardless of whether they had flowered or not. In this regard, some plants were harvested three times in the first year.



Yenice location (39°56'02.8"N 27°16'13.9"E) of Türkiye, 165 populations were identified from naturally occurring populations.

Samples were taken from the identified populations, and essential oil analyses were conducted, resulting in the identification of 41 populations.

Cuttings and seed samples were collected from the 41 populations and grown as seedlings in a greenhouse conditions.

Adaptation trials were conducted with the 41 different populations, and 39 populations with good adaptation were selected.

Based on the measured agronomic and quality traits, the five populations (A, B, C, D, and E) with the best characteristics were identified.

From the five identified populations (A, B, C, D, and E), the goal was to establish A clones through field studies based on the clonal selection method (Current Study)

◀**Fig. 2** Flowchart and relevant images illustrating the previous stages leading up to the current study with high OE content

In the second year of the experiment, necessary maintenance practices were carried out on time throughout the vegetation period, and observations and measurements were repeated for 2 years. Harvesting of the populations in the second year was conducted on 24 June and 7 July. After harvesting, essential oil analyses of the dried leaves of individual plants were performed in the Medicinal Plants Laboratories of the Field Crops Departments at Aydin Adnan Menderes University and Ege University throughout the experimental years.

Measurements

Plant height (cm): Individual plants within the studied populations were measured by recording the length of each plant from the soil surface to its tallest point at the harvest stage.

Fresh herb yield (g/plant): It was determined by harvesting each plant at a height of 10 cm above the soil surface and weighing it.

Dry herb yield (g/plant): It was measured by drying the harvested fresh herb in the shade at room temperature until a constant weight was reached (4–7 days) and then weighing it.

Dry leaf yield (g/plant): It was calculated by separating the leaves from the bulkier stems of the dried herb and then weighing the remaining sample (leaves, inflorescences and thin stems).

Essential oil content (%): It was determined volumetrically (ml/100 g) in dried leaves using a Neo-Clevenger apparatus. Essential oil content values were expressed as milliliters per 100 g of air-dried leaves (Wichtl 1971).

Statistical analysis

The findings were evaluated statistically using the SPSS software and TOTEMSTAT (Acikgoz et al. 2004) programs. Since the study was not designed as a replicated agronomic experiment, variance analysis was not performed. Instead, the data obtained for the selected traits of individual plants were evaluated using basic statistical methods. For each of the five populations, upper and lower limits, variance, standard deviation, and coefficient variation related to

measured parameters were determined. The graphs were prepared using the R software.

Result and discussions

The minimum and the maximum values obtained from the first, second, and non-flowering harvests of each population in the first year, and the minimum and maximum values for the second year, are summarized below for each parameter.

The measurements were done on successfully transplanted and healthy growing plants from populations A, B, C, D, and E. Population A had 702 transplanted plants, with 639 successfully developed. Population B, 581 of 648 seedlings grew well. Population C had 702 transplants, with 627 growing healthily by the second year. Population D had 636 seedlings, with 414 growing well and 90 flowered in the first year. Population E had 636 seedlings, with 117 flowered and 375 harvested regardless of flowering in the first year, followed by 408 harvested in the second year. None of the plants in populations D and E flowered for the 2nd harvest in the 1st year.

Based on agronomic observations of individual plants within the populations, plant height (cm) in the first year ranged from 10.0 to 70.0 cm in population A, 12.0–67.0 cm in population B, 7.0–61.0 cm in population C, 13.0–103.0 cm in population D, and 10.0–68.0 cm in population E. In the second year, plant height ranged from 14.0 to 92.0 cm in population A, 9.0–85.0 cm in population B, 10.0–90.0 cm in population C, 13.0–85.0 cm in population D, and 10.0–77.0 cm in population E.

All five populations exhibited variability in their plant height (cm) across different years and harvests. The second year showed increased plant height in some populations. Population A and Population D shows relatively consistent performance, while Population B and Population C displayed higher variability. Population E, although smaller in size compared to other populations, showed higher variation, particularly in the second year (Table 2). The average plant height (cm) values obtained in the study were found to be consistent with previous findings reported in the literature: Baytop (1999) recorded an average plant height of 60 cm; Zukauskas (2001) reported a range of 47–53 cm under natural conditions and 32–93 cm under cultivated conditions; Oflaz et al.

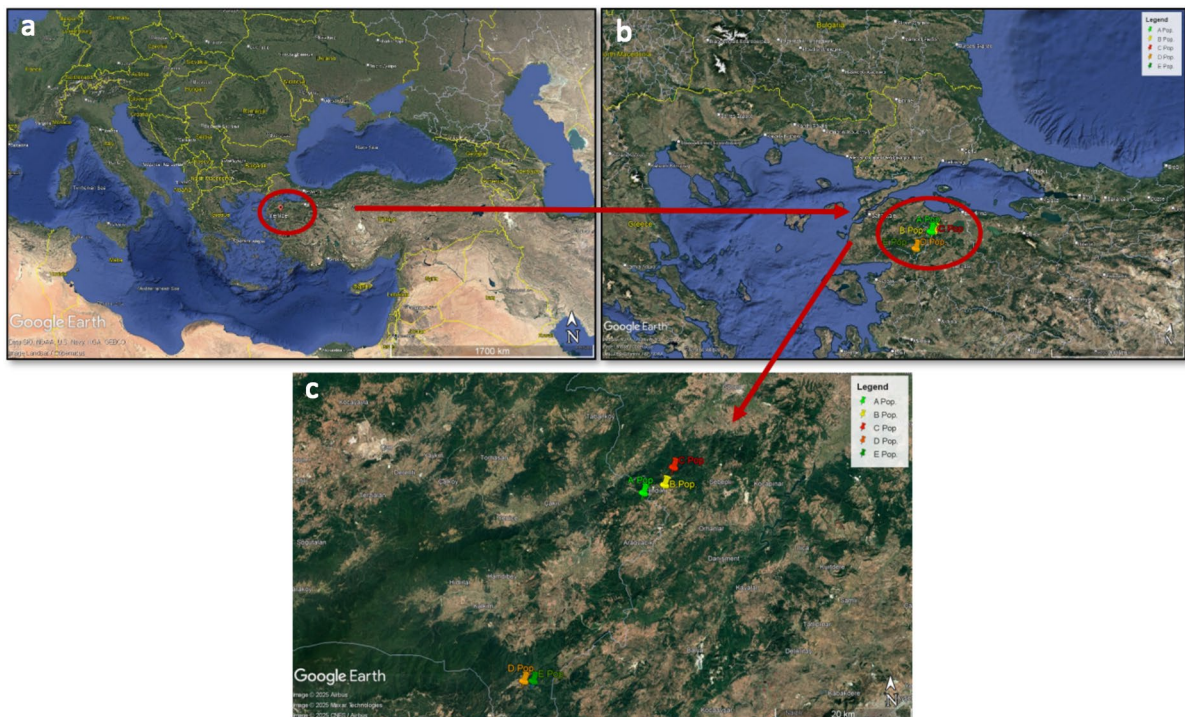
Table 1 Geographic coordinates of selected Istanbul Oregano (*Origanum vulgare* subsp. *hirtum*) populations in the yenice region of Türkiye

No	Population name	Coordinates (UTM, Zone 35)		Elevation
		x	y	
1	A	542,066	4,422,267	586
2	B	545,202	4,423,405	599
3	C	546,586	4,426,023	627
4	D	524,032	4,394,952	359
5	E	525,416	4,394,939	381

(2002) found plant heights ranging from 75 to 80 cm; Janke and De Armond (2004) documented values between 15.24 and 60.96 cm; W'glarz et al. (2006) recorded 54–68 cm; and Sarihan et al. (2006) reported a range of 25.4–70.6 cm. Additionally, Ahmad et al. (2008) found values ranging from 25.45 to 55.58 cm. Notably, the maximum plant height observed in our study is particularly comparable to the value reported by Zukauskas (2001) under cultivated conditions.

When examining the populations for fresh herb yield (g/plant), significant variation was observed both among populations and within individual plants, as observed when considering Table 2. The extent of the variation was considerable, with population A ranging from 4.0 to 767.0 g/plant, population B from 7.0 to 925.0 g/plant, population C from 5.0 to 758.0 g/plant, population D from 6.0 to 698.0 g/plant, and population E from 4.0 to 604.0 g/plant. These results clearly indicate a wide diversity in fresh herb yield across different populations and among individual plants within the same population. Furthermore, this differences also varied significantly depending on the experimental years and harvest. The average results over the two years revealed that the lowest fresh herb yield was observed in population E (78.9 g/plant), whereas the highest fresh herb yield was found in population A (176.6 g/plant). The findings showed an important range in fresh herb yield, which may be attributed to genetic diversity.

Leto and Salomone (1996) reported that the fresh herb yield of *Origanum vulgare* subsp. *hirtum* from 36 different populations of natural Sicilian origins

**Fig. 3** Distribution of selected Istanbul Oregano (*Origanum vulgare* subsp. *hirtum*) populations (A, B, C, D, and E) in the Yenice region of Türkiye. **a** Country overview, **b** Provincial overview, **c** Regional view

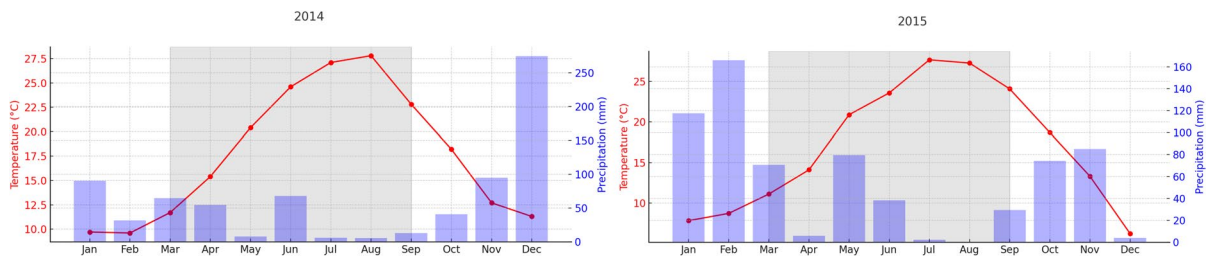


Fig. 4 Annual Precipitation, temperature, and relative humidity in the study area (shaded areas indicate active growth period of plant)

ranged from 210.2 to 2209.5 g/plant, and Sarrou et al. (2017) reported 770–2315 g/plant showing a considerable variation among populations. Similarly, Zukauskas (2001) documented substantial variability in herb yield among different populations of *Origanum* grown under natural and cultivated conditions in Latvia. Under natural conditions, yields ranged from 82 to 915 g/m², while in cultivated conditions, yields varied from 113 to 1050 g/m² in the first year, 125–2135 g/m² in the second year, and 516–2414 g/m² in the third year. The fresh herb yield values obtained in our study were found to be consistent with those reported by previous researchers. The variation we observed parallels that described by Leto and Salomone (1996) and Zukauskas (2001), suggesting that the fresh herb yield of the studied populations was within the typical range reported in the literature.

The dry herb yield (g/plant) values for individual plants of *Origanum vulgare* subsp. *hirtum* populations were presented in Tables 2. When examining values, it was observed that there was significant variation in dry herb yield among the populations across the two years. For population A, the dry herb yield ranged from 3.0 to 249.0 g/plant in the first year and from 2.0 to 301.0 g/plant in the second year, with a two-year average yield of 70.1 g/plant. In population B, the yield ranged from 2.0 to 262.0 g/plant in the first year and increased to 3.0–407.0 g/plant in the second year, with an overall average of 65.5 g/plant across both years. Similarly, population C exhibited yields between 3.0 and 236.0 g/plant in the first year and 2.0–302.0 g/plant in the second year, resulting in a two-year average of 58.8 g/plant. The population D, yields varied from 2.0 to 284.0 g/plant in the first year and 2.0–286.0 g/plant in the second year, the average of 55.7 g/plant over the two years. Population E showed lower yields compared to the other

populations, ranging from 2.0 to 206.0 g/plant in the first year and 3.0–183.0 g/plant in the second year, with a 2-year average of 31.9 g/plant. Population C had the highest average yields among non-flowering plants, this shows a strong vegetative growth performance. Population A and B both exhibited relatively consistent yield patterns among non-flowering plants, but with substantial variability in their second-year harvests. Population D displayed significant variability across all years. Population E showed the highest variability, especially in the second year. Sarrou et al. (2017) reported 382.9–806.7 g/plant and Leto and Salomone (1996) reported that the dry herb yield in natural populations ranged from 105.0 to 1100.0 g/plant. W'glarz et al. (2006) found values ranging from 243.1 to 636.6 g per plant. The findings from our study fall within the range reported by W'glarz et al. (2006), but are significantly lower than the maximum values reported by Leto and Salomone (1996). This indicates that, although our mean values are comparable to those reported in previous studies, certain genotypes exhibit significantly higher maximum values, clearly demonstrating their superior performance of dry herb yields. This variability is especially important for selecting the most superior genotypes within population.

The dry leaf yield of individual plants across the five populations was found to range from 0.4 to 171.0 g/plant. The highest individual plant dry leaf yield was recorded in population B during the second year (171.0 g/plant), followed by population C (156.0 g/plant) and population A (142.0 g/plant) in the same harvest cycle (Table 2). The two-year averages showed that the lowest and highest values were found in population E (20.4 g/plant) and population A (41.0 g/plant), respectively. These results highlight significant variation in dry leaf yield amongst

Table 2 *Origanum vulgare* subsp. *hirtum* populations A, B, C, D, and E, with mean, minimum and maximum values obtained from the first, second, and non-flowering harvests in the first year and mean, minimum and maximum values for the second year

Pop	Year	Har-vest	Plant	Plant height (cm)			Fresh herb yield (g/plant)			Dry herb yield (g/plant)			Dry leaf yield (g/plant)			Essential oil content (%)												
				Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV	Min	Max	Mean	SD	CV					
A	1	1st	180	17.0	68.0	35.7	7.6	21.3	13.0	325.0	71.5	46.2	64.6	3.0	121.0	25.4	18.6	73.1	1.1	77.1	18.0	12.1	67.3	1.5	7.7	4.6	1.0	21.8
	1	2nd	6	24.0	70.0	43.8	16.0	36.5	45.0	172.0	100.7	57.9	57.5	16.0	79.0	41.0	24.6	60.1	9.7	55.0	26.0	17.6	67.8	3.5	7.1	4.7	1.4	30.6
	1	Veg	606	10.0	68.0	29.4	10.9	37.1	27.0	702.0	235.8	111.4	47.3	7.0	249.0	89.3	41.4	46.4	3.5	136.0	44.7	21.3	47.6	0.4	3.4	1.3	0.4	28.4
B	2	Single	639	14.0	92.0	46.5	13.7	29.4	4.0	767.0	217.3	156.4	72.0	2.0	301.0	88.2	62.3	70.7	0.4	142.0	44.2	30.4	68.8	0.8	9.0	5.5	1.1	20.7
	1	1st	208	14.0	50.0	35.3	7.1	20.2	12.0	185.0	56.0	27.9	49.8	2.0	90.0	21.0	12.0	57.3	1.1	42.2	14.6	7.7	52.3	1.1	7.3	4.2	1.1	27.1
	1	2nd	11	28.0	67.0	44.3	13.4	30.2	17.0	200.0	97.1	70.4	72.5	8.0	83.0	40.5	29.3	72.5	4.0	47.6	24.2	17.7	72.9	3.0	4.7	3.7	0.6	14.8
C	1	Veg	530	12.0	67.0	32.4	9.7	29.9	20.0	699.0	229.9	107.7	46.8	4.0	262.0	88.8	41.0	46.2	3.3	120.0	41.1	19.9	48.4	0.2	2.5	1.3	0.4	29.3
	2	Single	581	9.0	85.0	40.7	14.8	36.3	7.0	925.0	208.1	159.6	76.7	3.0	407.0	81.0	63.5	78.4	1.6	171.0	37.8	27.4	72.6	1.8	8.5	5.0	1.0	19.5
	1	1st	190	18.0	60.0	36.9	7.6	20.6	10.0	187.0	52.9	28.1	53.1	3.0	77.0	23.1	12.0	52.0	2.0	48.5	15.3	8.1	52.9	1.0	7.6	4.5	1.0	22.9
D	1	2nd	4	33.0	50.0	44.3	7.7	17.4	20.0	102.0	51.5	35.2	68.4	9.0	42.0	21.8	14.2	65.1	4.5	28.2	14.0	10.1	71.6	2.8	4.6	3.5	0.8	23.2
	1	Veg	575	7.0	61.0	28.8	10.4	35.9	42.0	588.0	245.4	107.4	43.7	13.0	236.0	92.6	38.7	41.8	6.1	129.0	44.4	20.6	46.3	0.5	3.3	1.6	0.4	25.0
	2	Single	627	10.0	90.0	44.9	16.3	36.3	5.0	758.0	174.6	136.4	78.1	2.0	302.0	71.9	53.1	73.9	1.4	156.0	35.0	25.4	72.6	1.0	10.0	5.7	1.3	23.0
E	1	1st	90	20.0	88.0	35.8	8.7	24.4	7.0	162.0	41.9	27.5	65.6	2.0	54.0	17.2	10.5	60.7	1.5	37.7	10.9	6.8	62.2	1.1	6.9	4.4	1.0	22.8
	1	Veg	374	13.0	103.0	37.3	12.0	32.2	30.0	645.0	186.7	100.7	53.9	12.0	284.0	68.9	35.5	51.6	5.4	138.0	34.5	19.5	56.6	0.6	3.9	2.2	0.5	23.4
	2	Single	414	13.0	85.0	47.1	16.8	35.7	6.0	698.0	163.4	126.9	77.6	2.0	286.0	68.3	51.5	75.3	0.7	118.0	31.1	21.9	70.4	1.1	8.7	5.5	1.1	20.5
All	1	1st	117	17.00	60.00	36.1	8.9	24.6	5.0	146.0	36.6	24.1	65.8	2.0	57.0	14.7	9.7	66.1	0.8	37.6	9.3	6.2	67.1	1.5	8.6	4.1	1.1	27.0
	1	Veg	375	10.00	68.00	33.9	10.0	29.7	7.0	604.0	147.1	101.7	69.1	4.0	206.0	56.1	36.7	65.3	1.1	117.0	30.7	20.9	68.1	0.5	3.4	2.0	0.6	27.9
	2	Single	408	10.00	77.00	34.0	12.8	37.6	4.0	475.0	65.8	69.1	105.0	3.0	183.0	28.3	29.6	104.5	1.1	85.0	14.1	13.2	94.1	1.0	9.8	5.3	1.3	23.8
All	Year	Har-vest	Plant	Avr					Avr					Avr					Avr					Avr				
	1	1st	785	36.0					54.3					21.1					14.3					4.4				
	1	2nd	21	44.2					89.4					37.1					22.8					3.9				
	1	Veg	2460	31.8					215.8					81.8					40.2					1.6				
2	Single	2669	43.0					173.7					70.6					34.0					5.4					

Pop: population; **Min:** minimum, **Max:** maximum; **Veg:** unflowered plants (vegetative) harvest in November of the 1st year.

populations, with population A consistently outperforming others, particularly population E (Fig. 5).

A comparison of different harvest stages revealed that the vegetative harvest was generally the most productive across all populations, with mean dry leaf yields ranging from 30.7 to 44.7 g/plant, and maximum yields reaching 136.0 g/plant (Pop. A), 129.0 g/plant (Pop. C), and 120.0 g/plant (Pop. B). This likely reflects greater biomass accumulation prior to flowering. In contrast, the first harvests resulted in considerably lower yields, with population means ranging from 9.3 to 18.0 g/plant, likely due to early developmental stages and limited dry leaf mass. The second-year single harvests exhibited both high yield potential and high variability. For instance, while Populations B and C achieved the highest individual yields (171.0 and 156.0 g/plant, respectively), their coefficients of variation exceeded 72%, indicating significant heterogeneity among plants possibly due to genotypic differences at same population. These findings further indicate that the vegetative stage may be optimal for maximizing leaf biomass, but not necessarily ideal for achieving high essential oil content per gram of dry matter (Figs. 5 and 6). Additionally, the calculated dry leaf yields (g/m^2) show performance of certain populations. Population A achieved the highest yield with 419.5 g/m^2 , followed by Population B (361.9 g/m^2), Population C (360.3 g/m^2), Population D (205.8 g/m^2), and Population E (141.2 g/m^2).

To characterize the populations, essential oil content was measured for individual plants during harvests over the trial years. In the first year, the minimum and maximum essential oil percentages were determined as follows: 0.4–7.7% for Population A, 0.2–7.3% for Population B, 0.5–7.6% for Population C, 0.6–6.9% for Population D, and 0.5–8.6% for Population E. In the second year, the essential oil content values were generally higher compared to the first year. Specifically, the values ranged from 0.80 to 9.00% for Population A, 1.8–8.5% for Population B, 1.0–10.0% for Population C, 1.1–8.7% for Population D, and 1.0–9.8% for Population E. During the first year, the lowest value (0.2%) was observed in Population B, while the highest value (8.6%) was recorded in Population E. In the second year, the lowest value (0.8%) was observed in Population A, whereas the highest value (10.0%) was obtained from Population C (Table 2). The two-year average essential oil content ranged between 3.4 and 4.0%, with Population B having the lowest average value and Population D having the highest (Fig. 6).

The results revealed that 86% of the plants from population A contained an essential oil content ranging between 4.1 and 7.4%, while 71.4% of plants from population B had essential oil content between 3.8 and 5.8%. Additionally, 75% of plants in population C showed an essential oil content of 4.6–7.3%, whereas 82.1% of population D plants had an

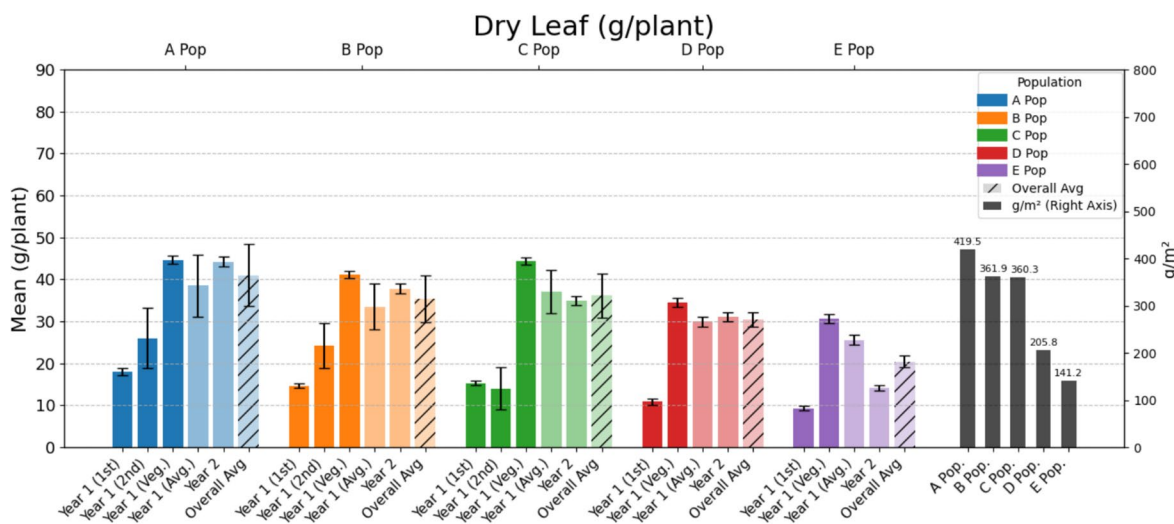


Fig. 5 Comparison of dry leaf yield (g/plant) across populations A, B, C, D, and E at different harvests (mean $\pm$ SE), and total production (g/m^2) for each population

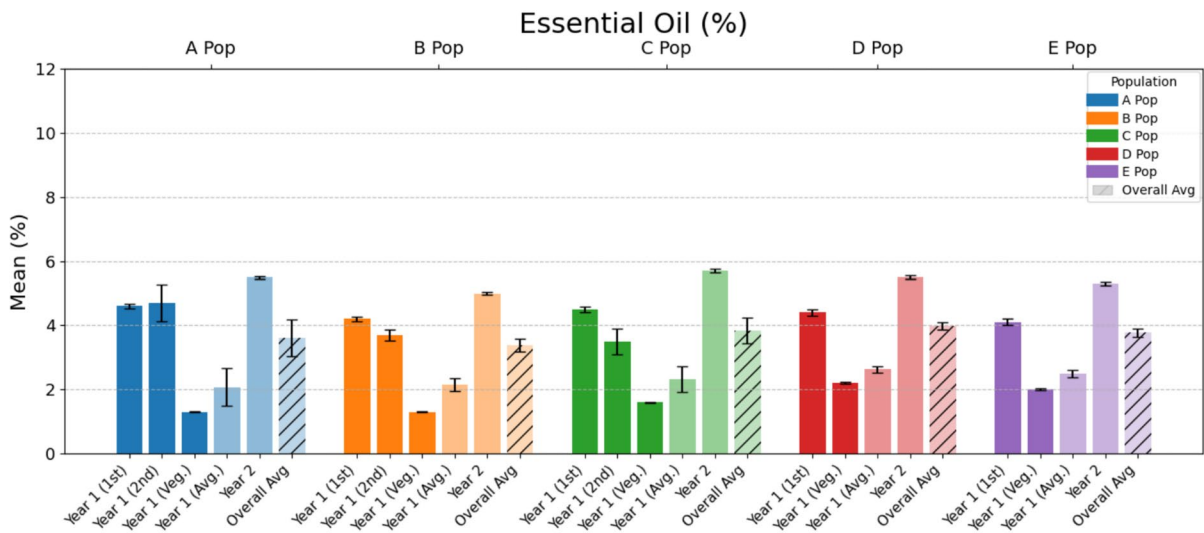


Fig. 6 Comparison of essential oil content (%) across populations A, B, C, D, and E at different harvests (mean $\pm$ SE)

essential oil content of 4.1–7.2%. Lastly, 86.3% of plants from population E exhibited an essential oil content between 3.6 and 7.2% (Fig. 7). These findings show substantial variability among populations in their essential oil content, indicating differences in the ability of each population to synthesize and accumulate essential oils. Figure 7 provides a visual representation of the range of essential oil percentages for each population, highlighting the variability and distribution within these different groups. The combined frequency distribution of essential oil content across populations A to E reveals a clear concentration of individuals within the moderate ranges. Most plants fall into the 3–5% and 5–8% categories, indicating that moderate essential oil levels are the most common. In contrast, individuals with less than 3% or greater than 8% essential oil content are relatively rare (Fig. 8).

Essential oil yield and quality vary significantly based on subspecies, environmental conditions, and breeding techniques (Alekseeva et al. 2020). Sarrou et al. (2017) reported between 6.0 and 11.1% and Tınmaz et al. (2002) found that the essential oil content in *Origanum vulgare* subsp. *hirtum* samples collected from natural habitats ranged between 1 and 6.1%. Leto and Salomone (1996) found a consistent 4% essential oil content in their natural habitat samples. Baytop (1999) reported a range of 4–5%, while Başer (2001) documented

values ranging from 1 to 7%. Zeybek and Zeybek (2002) noted 5%, and Oflaz et al. (2002) observed a range of 3.6–4.4%, including 3.9% for samples collected from Kazdağları. Kokkini et al. (1997) found essential oil contents ranging from 1.0 to 3.1% in plants collected from three different regions of Greece, whereas Russo et al. (1998) recorded values between 2.25 and 5.69% for plant samples collected from 24 populations in Italy. Veres et al. (2003) reported an essential oil content of 4.3% in *Origanum vulgare* subsp. *hirtum*. Sarıhan et al. (2006) found that under cultivation, the essential oil content in the first year ranged from 2.24 to 2.85%, increased to 2.75–3.20% in the second year, and further increased to 3.41–4.05% in the third year. W'glarz et al. (2006) observed oil contents ranging from 1.1 to 3.2% in individual plants. In cultivated plants, Karik et al. (2007) recorded oil contents between 6.17 and 6.71%, while Sancaktaroğlu and Bayram (2011) found a range of 3.78–4.59%. Karamanos et al. (2013) noted an increase in oil content per plant, with values of 1.5% in the second season and 2% in the third season. Lukas et al. (2015) analyzed the essential oil content of 502 individual plants from *Origanum vulgare*, originating from 51 populations across 17 countries, and found values ranging from 0.03 to 4.6%. Economou et al. (2011) observed essential oil contents between 5.5 and 10%

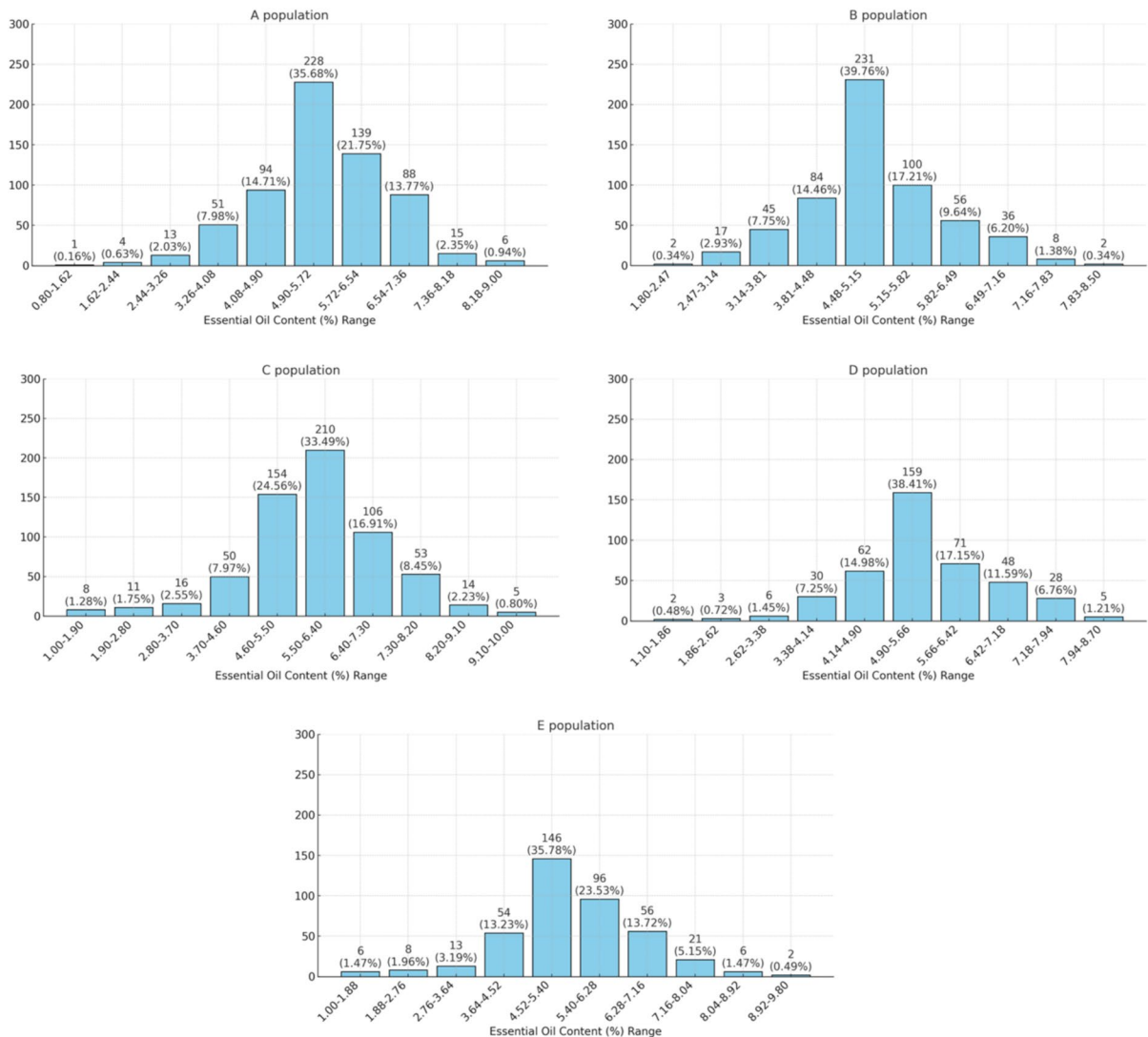


Fig. 7 Frequency distribution of essential oil content (%) in *Origanum vulgare* subsp. *hirtum* across different populations

in *Origanum vulgare* subsp. *hirtum*, and noted that their results were higher compared to those reported in all previous literature. Our two-year essential oil content results were within the range reported by Economou et al. (2011), with our maximum value being identical to their maximum. This outcome significantly exceeded the essential oil contents reported by other researchers, except as Sarrou et al. (2017) found maximum OE% (11,1%), higher than the 10% obtained in the present study, indicating a notable difference in oil production compared to previously documented studies.

Conclusions

In all populations, agronomic traits recorded during the first harvest of the first year were generally lower than those measured in the second and final (non-flowering) harvests of the same year. In contrast, essential oil content showed the opposite trend, peaking during the first harvest of the first year compared to the other harvests. Additionally, when comparing the results of the first and second years, a noticeable increase was observed in plant height, fresh herb yield, dry herb yield, dry leaf yield, and essential oil content in the second year. This increase can

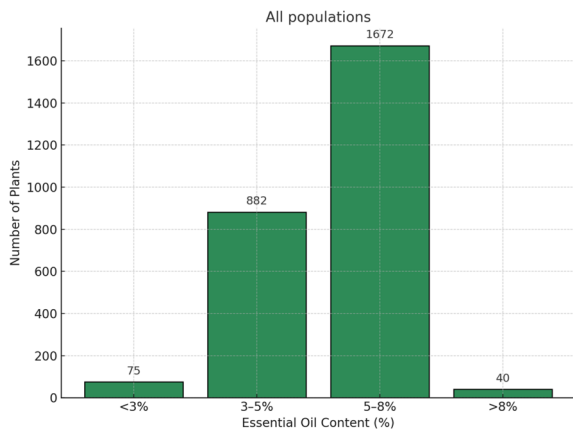


Fig. 8 Distributions of essential oil content (%) in *Origanum vulgare* subsp. *hirtum* within all populations

be attributed to the improved root development and extended vegetation period starting from the second year in perennial plants, which accelerated the growth of the above-ground parts, thereby enhancing yield. In populations D and E, a second harvest could not be performed after the first harvest due to lack of flowering; however, this issue was limited to the first year. Since the first year typically represents establishment phase in perennial plants characterized by lower yield and quality, this regrowth limitation is considered to have limited practical significance. Overall, the findings indicate a broad genetic diversity within the populations. Significant differences in yield and quality were identified among genotypes. Agronomic and quality evaluations of populations A, B, C, D, and E revealed considerable variation both within and among the populations. Given the wide range of minimum and maximum values, it can be inferred that the genetic variability for breeding purposes is quite promising. Based on the results, genotypes with high yield and essential oil content values were used for further selection, aiming to produce varieties with superior traits. Especially results showed that some *Origanum vulgare* subsp. *hirtum* individual plants within the populations exhibited essential oil content 10%, significantly above the average. This holds great value for both agricultural and medicinal applications. The A clones (next stage) were planned by selecting 129 genotypes from population A, 136 from population B, 143 from population C, 128 from population D, and 105 from population E. The clone

selection breeding cycle is expected to be completed through future studies, requiring further research for variety development.

Acknowledgements This work was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) (Grant number 113O285). We would like to express our gratitude to TÜBİTAK for their financial support.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by O.A. U.T., E.B., and Ç.S. The first draft of the manuscript was written by O.A. and U.T. Figures were prepared by U.T.; all authors reviewed and approved the final manuscript.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). This work was supported by the Scientific and Technological Research Council of Türkiye (TÜBİTAK) (Grant number 113O285). We would like to express our gratitude to TÜBİTAK for their financial support.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Populations A and B were presented separately at different conferences as poster and publish in a special issue of conference with limited details. However, in this study first time, all five populations (A, B, C, D, and E) were comprehensively evaluated, and their relationships were analyzed in detail.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Acikgoz N, Ilker E and Gokcol, A (2004) Evaluation of biological research on the computer. E.U. TOTEM No: 2.

- SPSS 17.0. 2008. SPSS 17.0 for Windows. Chicago, IL: SPSS Inc
- Ahmad S, Koukab S, Islam M, Ahmad K, Aslan S, Aminullah GA (2008) Germplasm evaluation of medicinal and aromatic plants in highland Balochistan, Pakistan. *Pak J Bot* 40:1473–1479
- Alekseeva M, Zagorcheva T, Atanassov I, Rusanov, K (2020) *Origanum vulgare* L. A review on genetic diversity, cultivation, biological activities and perspectives for molecular breeding. *Bulg J Agricult Sci* 26(6):1203–1210
- Arabacı O (2022) Selection breeding in Istanbul oregano (*Origanum vulgare* subsp. *hirtum*) populations. In: Tünctürk R (ed) Functional applications, trade, and sustainability of medicinal and aromatic plants. Iksad Press, Ankara, pp 3–54 (in Turkish)
- Başer KHC (1993) Essential oils of anatolian Labiatae. *Acta Hort* 333:217–238
- Başer KHC (2001) Oregano, a plant of many virtues. *J Sci Technol* 402:74–77 (in Turkish)
- Başer KHC, Özek T, Tümen G, Sezik E (1993) Composition of the essential oils of Turkish *Origanum* species with commercial importance. *J Essent Oil Res* 5:619–623
- Baytop T (1999) Therapy with medicinal plants in Turkey, past and present (2nd print with additions). Nobel Tıp Kitapevleri, p. 480. (in Turkish).
- Birinci S (2008) Beneficial plants naturally found in Eastern Black Sea region and their usage fields. MSc Thesis, Cukurova University, Institute of Natural and Applied Sciences Department of Field Crops, Adana. (in Turkish)
- Davis PH (1982) Flora of Turkey and the East Aegean Islands, vol 7. Edinburgh University Press, Edinburgh, pp 308–309
- Demir İ, Turgut İ (1999) General plant breeding. Ege University Faculty of Agriculture Publications, No. 496, İzmir (in Turkish)
- Economou G, Panagopoulou G, Tarantilis P, Kalivas D, Kotoulas V, Travlos IS, Polysioub M, Karamanos A (2011) Variability in essential oil content and composition of *Origanum hirtum* L., *Origanum onites* L., *Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from the Greek island Ikaria. *Ind Crops Prod* 33:236–241
- Esen G, Azaz A, Kurcuoglu M, Başer K, Tınmaz A (2007) Essential oil and antimicrobial activity of wild and cultivated *Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart from the Marmara region, Turkey. *Flavour Fragr J* 22:371–376. <https://doi.org/10.1002/FFJ.1808>
- Ferrer M, Good-Avila S, Montaña C, Domínguez C, Eguarte L (2009) Effect of variation in self-incompatibility on pollen limitation and inbreeding depression in *Flourensia cernua* (Asteraceae) scrubs of contrasting density. *Ann Bot* 103(7):1077–1089. <https://doi.org/10.1093/aob/mcp033>
- Hoffman W, Mudra A, Plarre W (1971) Lehrbuch der züchtung landwirtschaftlicher Kulturpflanzen. Band 1. Verlag Paul Parey, Berlin, pp 51–53
- Janke R, De Armond J (2004) Oregano, *Origanum vulgare*. Kansas State University Agricultural Experiment Station and Cooperative Extension Service, MF-2621
- Karamanos AJ, Sotiropoulou DEK (2013) Field studies of nitrogen application on Greek oregano (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart) essential oil during two cultivation seasons. *Ind Crops Prod* 46:246–252
- Karık Ü, Tınmaz AB, Kürkçüoğlu M, Başer KHC, Tümen G (2007) Effect of different cutting time on yield and quality in Istanbul oregano (*Origanum vulgare* L. subsp. *hirtum*) populations. *Bahçe* 36:37–48 (in Turkish)
- Kitıkı A, Sarı O, Oğuz B, Ceylan A, Bayram E, Özyaz N (1997) The identification of superior types amongst Western Anatolian oregano (*Origanum onites* L.) populations and determining their progress under culture. Report of Results, T.C. Ministry of Agriculture, Ege Agricultural Research Institute, Menemen-Izmir. (in Turkish)
- Kokkini S, Karausou R, Derdioti A, Kriges N, Lanaras T (1997) Autumn essential oils of Greek oregano. *Phytochemistry* 44:883–886
- Leto C, Salamone A (1996) Bio-agronomical behaviour in Sicilian *Origanum* ecotypes. In: Promoting the conservation and use of under-utilized and neglected crops. 14. Oregano. Proceedings of the IPGRI International Workshop on Oregano, 8–12 May. CIHEAM, Valenzano, Bari, Italy
- Lukas B, Schmiederer C, Novak J (2015) Essential oil diversity of European *Origanum vulgare* L. (Lamiaceae). *Phytochemistry* 119:32–40
- Nasrallah J, Nasrallah M (1993) Pollen[mdash]Stigma signaling in the sporophytic self-incompatibility response. *Plant Cell* 5:1325–1335. <https://doi.org/10.1105/tpc.5.10.1325>
- Oflaz S, Kürkçüoğlu M, Başer KHC (2002) *Origanum onites* ve *Origanum vulgare* subsp. *hirtum* Üzerinde Farmakognozik Araştırmalar, 14. Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 29–31 Mayıs, Eskişehir, Eds. K.H.C. Başer ve N. Kırimer, ISBN 975-94077-28.
- Russo M, Galletti GC, Bocchini P, Carnacini A (1998) Essential oil composition of wild populations of Italian oregano spice (*Origanum vulgare* L. subsp. *hirtum* (Link) Ietswaart): a preliminary evaluation of their use in chemotaxonomy by cluster analysis of inflorescences. *J Agric Food Chem* 46:3741–3746
- Sancaktaroglu S, Bayram B (2011) Researches on the determination of the yields and quality characteristics of Istanbul oregano (*Origanum vulgare* subsp. *hirtum* L.) populations of different origin. *Ege Univ Ziraat Fak Derg* 48:265–276
- Sarıhan EO, İpek A, Arslan N, Gürbüz B (2006) The effects of different row spacing and intra-row spacing on yield and yield components of oregano (*Origanum vulgare* var. *hirtum*). *J Agricult Sci Ankara Üniv Fac Agricult* 12:246–251
- Sarrou E, Tsivelika N, Chatzopoulou P, Tsakalidis G, Menexes G, Mavromatis A (2017) Conventional breeding of Greek oregano (*Origanum vulgare* ssp. *hirtum*) and development of improved cultivars for yield potential and essential oil quality. *Euphytica* 213:1–16
- Tınmaz AB, Kürkçüoğlu M, Başer KHC, Öztürk M (2002) Determination of quality characteristics of Istanbul oregano (*Origanum vulgare* subsp. *hirtum*) populations from the Marmara region. In: Proceedings of the 14th meeting of plant drug raw materials, 29–31 May, Eskişehir. ISBN: 9759407728. (in Turkish)
- Tuğlu Ü, Baydar H (2019) Tıbbi adaçayı (*Salvia officinalis* L.)'nda klon seleksiyonu ile geliştirilmiş b-klonlarının tarımsal ve teknolojik özellikleri. *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 23(2):452–456. <https://doi.org/10.19113/sdufenbed.514564>

- Veres K, Varga E, Dobos Á, Hajdú Z, Máthé I, Németh E, Szabo K (2003) Investigation of the composition and stability of the essential oils of *Origanum vulgare* ssp. *vulgare* L. and *O. vulgare* ssp. *hirtum* (Link) Letswaart. *Chromatographia* 57:95–98
- W'glarz Z, Osidska E, Geszprych A, Przybyb J (2006) Intraspecific variability of wild marjoram (*Origanum vulgare* L.) naturally occurring in Poland. *Rev Bras Pl Med Botucatu, Poland* 8(Special Issue):23–26
- Wichtl M (1971) Die pharmakognostisch-chemische Analys Band 12. *Franfurt*, p. 479
- Zeybek U, Zeybek N (2002) Systematics and important aspects of pharmaceutical botanics, plants with hidden seeds (Angiospermae). Ege University Publishing, pp. 383–385, Bornova, Izmir. (in Turkish)
- Žukauska I (2001) Agrobotanical features and productivity of wild and cultivated populations of *Origanum vulgare*. *Bot Acta Biol Daugavp* 1:107–109

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.