



## Effect of Ecosystem Type and Harvesting Time on Morphological Indicators and Biochemical Characteristics of *Thymus kotschyanus* Iran

F. Bahadori<sup>1</sup> M.R. Alipour<sup>2</sup> M.K. Kianian<sup>3</sup>

<sup>1</sup>Assistant Professor of Research Division of Natural Resources Department, Semnan Agricultural and Natural Resources Research and Education Center, AREEO, Semnan, Iran.

<sup>2</sup>Graduate student in Horticultural Sciences, Save university, Iran.

<sup>3</sup>Assistant Professor of Desert Studies Faculty, Semnan University, Iran.  
m\_kianian@semnan.ac.ir

Corresponding author: farbahadori@gmail.com

### Abstract:

In natural and agricultural ecosystems, factors such as humidity, water, nutrients, light and altitude are among the basic factors that determine the quantity and quality of plants. Thyme is one of the most important medicinal plants from the mint family (Lamiaceae). The current research was carried out based on the split plot design in the form of randomized complete blocks and in 3 replications at the Agricultural and Natural Resources Research Center of Semnan province. The treatments included two types of natural and agricultural ecosystems and two harvesting times before flowering and full flowering which had their effect on the morphological indicators, biochemical characteristics and the quantity and quality of the essential oil of the medicinal plant of mountain thyme (*Thymus kotschyanus*) was investigated. According to the results obtained from this experiment, it was found that the effect of different ecosystems and different harvest times on the tested factors are statistically significant at 1% and 5% levels. All biochemical characteristics including chlorophyll a, chlorophyll b, chlorophyll T, carotenoid, anthocyanin and flavonoid showed higher levels in the natural ecosystem, except for chlorophyll a/b, which was higher in the agricultural ecosystem, and most of the biochemical characteristics during Flowering was more except for chlorophyll a/b, anthocyanin and flavonoid which were more flowering at the earlier time. All the morphological indicators, including fresh and dry weight of the whole plant, height of aerial and underground organs, large and small diameter of the plant, plant circumference and dry yield per hectare at the time of full flowering and in the agricultural ecosystem, were more except the fresh weight of leaves and leaf area which They were more in the natural ecosystem. Also, the percentage of essential oil was the highest at the time of full flowering and in the agricultural ecosystem, but the essential oil compounds showed a higher percentage at the time of full flowering and in the natural ecosystem. But the amount of total phenol was not affected by the test treatments and no significant difference was observed between the obtained results.

### Keywords:

chlorophyll, carotenoid, anthocyanin, flavonoid and phenol.

## Introduction

The science of knowing the therapeutic effects of plants is one of the oldest known human sciences. The plateau of Iran has more than 7500 species of plants, many of which contain medicinal value and metabolic reserves. Thyme is a plant native to Asia and Europe, which includes 400 species in the world and 17 species in Iran, of which 14 species, including mountain thyme (*Thymus kotschyanus*), are native to Iran. Thyme, due to the presence of high effective substances in the cosmetics and health industries, food industries and modern pharmaceutical industries, due to the presence of more than 28 types of different chemical compounds, including thymol, linalool, alpha-terpinene, alpha-pinene and carvacrol, are used in the preparation of various drugs (Carrapiso et al., 2020). Thyme essential oil is a colorless or dark yellow or reddishbrown liquid. And it has a pleasant, strong smell and a spicy taste, it dissolves in alcohol and fatty oils and decomposes in the vicinity of light, and its specific gravity is between 0.915 and 0.935 (Askari et al., 2023). Since the quantity and quality of the effective substances of thyme are different in different stages of plant growth and climatic conditions, ecosystem and different harvest times, and it plays a major role in the growth and production rate of its effective substance, therefore, the purpose of this research is to investigate the effect The ecosystem and different harvesting times are on the morphological, physiological, quantity and quality of the essential oil of mountain thyme (*Thymus kotschyanus*), so that according to it and by choosing the right ecosystem and harvesting time, it is possible to obtain the desired morphological and physiological indicators and The maximum amount of essential oil was achieved with the appropriate quantity and quality (Iftikharet al., 2023; Elahianet al., 2021; Stahl and Saez, 2002). Due to the growing value of the thyme plant, in the past years, extensive research on thyme has been started in different countries of the world, including Iran, which is still ongoing, but its cultivation in our country is still done on an experimental basis. And its production and exploitation depends on natural habitats located in pastures and forests. By collecting the genetic resources of this plant and producing it in suitable agricultural conditions, it is possible to increase the added value and produce higher quality products. Therefore, according to the research conducted regarding the determination of environmental and ecological factors on the effective medicinal substance of the mountain thyme plant (*Thymus kotschyanus*), it led to a study and research on this issue.

## Materials and methods

This research was carried out in the research farm of Jihad Agricultural Extension Service Center with an area of 10 hectares located in Shahmirzad section of Mahdishahr city in Semnan province in the form of a completely randomized block design with three replications in the form of openair cultivation. took Also, the origin and botanical characteristics of the thyme plant in the natural ecosystem were also considered in the heights of Jashloubar, located in the north of Shahmirzad, which was the main habitat of mountain thyme in Semnan province. Therefore, the seeds were prepared from the above-mentioned area and planted in the open-air tank and in light soil, in rows with a distance of 30 cm and the intervals on the row of 5 cm and a depth of 0.5 cm. After, they were transferred to the main land and planted with a distance between the rows of 40 cm and the distance on the row of 30 cm. Before carrying out the planting operation in the area, it was

taken from the soil and sent to the laboratory to check the physical and chemical properties of the soil (Table 1).

Table 1. Soil analysis results of agricultural and natural ecosystems

Ecosystem	Depth(cm)	soil pattern	N%	P(ppm)	K(ppm)	Acidity(PH)	EC	OC(%)	CA-MN(mql/L)	Na(mql/L)
Agricultural	0-30	Silty loam	0.04	25	320	7.7	0.42	0.45	2.3	3.1
Natural		sandy loam	0.07	15	200	7.9	0.57	0.75	3.4	2.5

### Harvest time

According to the purpose of the experiment, the plants were harvested at the desired times as follows:

#### -Harvesting plants before flowering

After the plants have grown enough and before they enter the reproductive (flowering) phase, i.e. on the 15th of May 2018, the entire vegetative body of 10 plants was harvested from 10 cm of the ground surface with gardening shears and their fresh weight was measured. And the harvested plants were labeled. On the label, the harvesting stage and the date of harvesting of the plant were mentioned. Then they were placed in the plant drying hall at Semnan Agricultural and Natural Resources Research Center to dry in the shade and under open air flow. After a week, the plants were dried and their dry weight was measured.

#### -Harvest plants in full flowering stage

After the plants entered the reproductive phase (full flowering) i.e. 80% of their flowers had opened, they were harvested and their fresh weight was measured like the previous plants, and the harvest stage and harvest date were noted on their labels. Then they were placed in the drying room and after drying completely, their dry weight was obtained. Because the flowers of mountain thyme were observed in various colors, plants were harvested at this stage from flowers with purple and white flower colors, which included the highest percentage. In this experiment, extraction of essential oil and other biochemical compounds of mountain thyme including chlorophyll, carotenoid, flavonoid, anthocyanin and total phenol and evaluation of morphological indicators including fresh and dry weight of the whole plant, fresh and dry weight of roots, fresh and dry weight of shoots, weight Leaf wet and dry, root length and stem height were measured in the laboratory of Semnan Agricultural and Natural Resources Research Center and essential oil analysis was done by gas chromatography (GC) in Damghan Islamic Azad University.

#### -Measurement of plant pigments

The content of chlorophyll a, b and carotenoid was calculated by the method of Lichtenthaler (1987) and using the following relationships.

$$Ca = 12/25(A\ 663) - 2/79(A\ 647)$$

$$Cb = 21/50(A\ 647) - 5/10(A\ 663)$$

$$C(x+c) = (1000(A\ 470) - 1/82(ca) - 85/02(cb))/198$$

In this formula, ca, cb and c(x+c) are chlorophyll a, chlorophyll b and carotenoids, respectively. The concentration was determined in mg/ml of plant extract. In order to

measure anthocyanins, the amount of 100 mg of fresh thyme leaves from each treatment was carefully weighed with 3 repetitions and well ground with 5 ml of acidic methanol solution (methyl alcohol and hydrochloric acid in a ratio of 99:1) and the resulting extract was centrifuged and The solution was placed in the dark for 1 night, and then its absorbance was read at a wavelength of 550 nm by a spectrophotometer

To calculate the concentration of anthocyanins, the extinction coefficient equal to 33000 mm<sup>-1</sup> cm<sup>-1</sup> was used (krizek et al., 1998). To measure the amount of flavonoids, 100 mg of fresh thyme leaves from each treatment were carefully weighed with 3 repetitions and with 5 ml of well ground acidic ethanol solution and the resulting extract was centrifuged and the supernatant solution was slowly placed in a spa bath for 10 minutes. It was heated at a temperature of 80 degrees Celsius and then its absorbance was read by a spectrophotometer at 3 wavelengths of 330, 300 and 270 nm. (Krizek et al., 1998). In order to measure the total amount of phenol, 100 mg of fresh thyme leaves from each treatment were carefully weighed with 3 repetitions and ground well with 5 ml of 95% ethanol and kept in the dark for 24-72 hours. Then add 1 cc of 95% ethanol to 1 ml of the supernatant solution and distill the solution twice with distilled water to 5 ml, then add 0.5 cc of Folin reagent 50% and 1 cc of 5% calcium carbonate. becameThe resulting mixture was kept in the dark for 1 hour and then its absorbance at 725 nm was read by a spectrophotometer (Gao et al., 2000).

In order to extract the essential oil of the plant, it was done in the laboratory of the Semnan Agricultural Research Center using the distillation method with water using a Cloninger machine. An essential oil extractor is a glass device whose basis is distillation with water.

The procedure for extracting the essential oils of plants is as follows: a 100-gram sample of dried thyme parts of each plant was powdered and placed in a flask with 400 ml of distilled water and heated for 2 hours. As a result of heat, the pressure of water vapor increases and essential oil enters the cooler along with water vapor. Condensation has taken place in the cooler and the drops of essential oil in the water move towards the graduated tube in two distinct phases. In other words, when the mixture of water vapors and essential oil reaches an environment with lower air pressure, because the condensation point of water is not the same as the condensation point of essential oil, then they are in two different phases. Because thyme essential oil is light, it accumulates on the surface of the water and the excess water goes down through the connecting tube. It is possible to measure the volume of essential oil in the graduated tube.

At the end, the essential oil obtained from each plant was collected inside a special glass container and weighed with an accuracy of 0.001 grams, and all three repetitions related to one treatment were collected inside a glass container. Dry sodium sulfate was used to remove water from essential oil. Because the presence of water hydrolyzes the esters in the essential oil and turns them into acid and alcohol, and it must be stored in cool and dark conditions of the refrigerator. Since the amount of essential oil is measured in the dry weight of the organs, therefore the amount of moisture of the organs should be obtained during the extraction of the essential oil, for this purpose, 5 samples of 5 grams of the uniform mass of the organs in the oven with a temperature of 105 degrees Celsius for 3 The clock was set. Then, using the existing relationships, the humidity level of the organs was determined to be 5%.

### **-Measurement of morphological indicators**

The morphological characteristics of the plant, including the fresh and dry weight of the whole plant, were measured by an accurate laboratory scale with an accuracy of 0.001. Also, the length of aerial and underground organs, plant diameter and plant circumference were calculated based on centimeters and leaf surface was calculated based on square centimetres.

### **-Essential oil analysis**

After extracting the essential oil, isolation and identification of essential oil compounds were done in the chemical laboratory of Islamic Azad University, Damghan branch. In order to investigate the constituents of the essential oils of the plants harvested in the ecosystem and at different harvesting times, the samples of 3 repetitions of each treatment were mixed with each other for analysis with a gas chromatography (GC) machine. Therefore, due to the fact that the error rate of the device in essential oil analysis is very small, the average of each composition of the essential oil is obtained for each treatment. The specifications of the gas chromatography device used in this research are as follows:

Name of the device: gas chromatograph VARIAN CP-3800;

The dimensions of the DB-5 column: the length of the column is 30 meters and the inner diameter of the column is 0.32 mm;

The thickness of the stationary phase layer: 0.25 micrometers;

Column temperature programming: from 50 to 240 °C with a temperature increase of 2.5 °C per minute, and stop at 100 °C for 10 minutes;

Injection chamber temperature: 290 degrees Celsius

Detector type: Detector FID

Carrier gas: N<sub>2</sub> nitrogen with a purity of 99.999% at a speed of 0.8 mm/min.

### **-Statistical model of data analysis**

In this research, the experiment was conducted based on the split plot design in place in the form of randomized complete blocks and the measurements were carried out in 3 replications. The statistical data obtained from the measurements were analyzed by MSTC software and graphs were drawn using Excel software. Before analyzing the data, their normality was determined by Ln and Log methods, and after confirming the normal distribution mode, they were analyzed. The mean data were compared with Duncan's multi-range test.

### **Results and discussion**

In this research, the effect of ecosystem type and harvesting time on morphological indicators and biochemical parameters and the quantitative and qualitative performance of mountain thyme essential oil was investigated. Variables such as the amount of chlorophyll b and a, carotenoids, anthocyanins, flavonoids and total phenol, as well as the fresh and dry weight of the whole plant, the length of the aerial and underground parts of the plant, the diameter of the plant, the environment of the plant, as well as the percentage of essential oil and compounds in The essential oil was measured. All the biochemical, morphological, physiological, quantity and quality characteristics of the mountain thyme plant under the influence of the tested treatments (ecosystem type and plant harvesting time) are described below. which is described below:

ns: non-significant difference at the 5% probability level

:\*significant difference at the 5% probability level



:\*\*highly significant difference at the 1% probability level

According to the variance analysis table (Table 1-4), the results showed that there was a significant difference between the treatments and the comparison of the average data in different ecosystems and different harvest times and their interaction was done and at each of the same levels in The probability level of 1% and 5% was checked (Tables 2 and 3).

Table 2. Variance analysis of the effect of different ecosystems and different harvest times on the morphological indicators of mountain thyme

Sources of Variation	df	Mean Square							
		Plant wet weight	dry weight of plant	wet weight of underground organs	dry weight of underground organs	wet weight of aerial organs	dry weight of aerial organs	fresh weight of leaves	dry weight of leaves
place	1	ns60.53	ns7/65	ns24/5	ns1/69	ns17/32	ns23/85	*0/77	*0/02
error	4	82.04	18/71	26/67	5/93	37/66	3/35	0/60	0/01
harvest time	2	**1375/45	**418/05	**3882/20	**145/27	*346/05	*49/18	ns3/34	**0/23
Interaction place*time	2	**1118/61	**317/63	**198/13	**151/73	**483/25	ns24/45	*6/23	**0/29
error	8	66/24	12/04	13/492	3/06	48/92	6/41	0/75	0/008

Table 3. Variance analysis of the effect of different ecosystems and different harvest times on the morphological indices of mountain thyme

Sources of Variation	df	Mean Square						
		root length	Bush height	leaf surface )cm(	large diameter	large diameter	plant environment )cm(	dry weight yield(kg)
place	1	*143/37	**86/68	*46/72	ns12/5	ns53/39	ns180/5	**4272124/5
error	4	9/1	1/51	5/7	18/28	21/72	185/78	9493/3
harvest time	2	ns9/75	**4/06	*52/87	**239/39	**165/06	**1795/06	*241169/49
Interaction place*time	2	ns5/7	ns0/06	**105/17	**243/17	**178/72	**1991/17	*220716/46
error	8	19/25	0/26	8/04	19/36	15/97	158/69	29271/86

The results of the morphological measurement of the thyme plant, after sampling two fresh weights at the beginning of sampling and then after drying in the open air, showed that the highest fresh weight and dry weight of the whole plant (including aerial and underground organs) at the time of harvest The full flowering of thyme plant with white flowers in the agricultural ecosystem was 80.8 and 40.71 grams, respectively, and the lowest weight before flowering in the said ecosystem was 37.36 and 15.01 grams, respectively. By measuring the fresh and dry weight of thyme leaves, the highest fresh weight of the leaves was 6.08 grams in the natural ecosystem at the time of full flowering of the purple flower and the highest dry weight of the leaves in the cultivated ecosystem was observed at the time of full flowering of the white flower at the rate of 1.34 grams. Table 4).

The results of other investigated parameters of the morphological characteristics of the thyme plant in both agricultural and natural ecosystems during different harvest times showed that

the largest leaf area in the natural ecosystem at the time of harvesting the full flowering of the purple flower was 25.47 cm, the largest diameter of the plant (The largest and the smallest diameter) are 35.67 and 33.67 cm, respectively, the larger diameter in the natural ecosystem at the time of harvesting the full flowering of the purple flower and the smaller diameter in the agricultural ecosystem at the time of the harvesting of the full flowering of the white flower. Also, by estimating the environment of thyme plant, the maximum and minimum environment at the time of full flowering of white flower in the agricultural ecosystem were 114.67 and 58 cm, respectively. By examining the yield of dry weight of thyme plant per hectare, the highest amount of dry weight of 1313.91 kg was observed in the agricultural ecosystem and the lowest amount of dry weight of the plant in the natural ecosystem was 102.42 kg (Table 5).

Table 4. Comparison of the average effects of harvesting time and ecosystem type on the morphological indices of mountain thyme

Check location	harvest time	Plant fresh weight) gr(	Dry weight of plant)gr(	root fresh weight) gr(	Root dry weight) gr(	shoot fresh weight)g r(	leaf fresh weight )gr(	Leaf dry weight)g r(
agricultural	before flowering	/3736 <sup>c</sup>	15/01 <sup>c</sup>	14/57 <sup>b</sup>	7/6 <sup>b</sup>	22/79 <sup>c</sup>	3/17 <sup>c</sup>	0/78 <sup>b</sup>
	Full bloom of purple flowers	55/79 <sup>b</sup>	21/58 <sup>b</sup>	22/02 <sup>bc</sup>	6/54 <sup>c</sup>	33/77 <sup>bc</sup>	3/75 <sup>b</sup>	0/73 <sup>b</sup>
	Full bloom of white flowers	80/8 <sup>a</sup>	40/71 <sup>a</sup>	37/11 <sup>a</sup>	21/86 <sup>a</sup>	47/02 <sup>a</sup>	5/46 <sup>ab</sup>	1/34 <sup>a</sup>
Natural	before flowering	40/03 <sup>bc</sup>	16/76 <sup>bc</sup>	14/16 <sup>c</sup>	7/43 <sup>b</sup>	27/32 <sup>b</sup>	3/81 <sup>bc</sup>	0/64 <sup>c</sup>
	Full bloom of purple flowers	75/71 <sup>ab</sup>	33/05 <sup>ab</sup>	30/1 <sup>ab</sup>	17/58 <sup>ab</sup>	45/61 <sup>ab</sup>	6/08 <sup>a</sup>	1/12 <sup>ab</sup>
	Full bloom of white flowers	<sup>bc</sup> 47/21	23/57 <sup>b</sup>	22/45 <sup>bc</sup>	12/83 <sup>bc</sup>	24/76 <sup>b</sup>	3/73 <sup>b</sup>	0/86 <sup>bc</sup>

Table 5. Comparison of the average effects of harvesting time and ecosystem type on the morphological indices of mountain thyme

place	harvest time	leaf surface(cm)	large diameter	large diameter	plant environment(cm)	dry weight yield(kg)
Agricultural	before flowering	12/43 <sup>C</sup>	19/67 <sup>C</sup>	16/67 <sup>C</sup>	58 <sup>C</sup>	633/91 <sup>bc</sup>
	Full bloom of purple flowers	13/37 <sup>b</sup>	27/33 <sup>bc</sup>	23 <sup>b</sup>	77/67 <sup>b</sup>	1313/91 <sup>a</sup>
	Full bloom of white flowers	18/9 <sup>ab</sup>	40/33 <sup>a</sup>	33/67 <sup>a</sup>	114/67 <sup>a</sup>	1312/56 <sup>ab</sup>
Natural	before flowering	14/53 <sup>bc</sup>	22/33 <sup>b</sup>	16/67 <sup>C</sup>	62/67 <sup>bc</sup>	102/42 <sup>bc</sup>
	Full bloom of purple flowers	25/47 <sup>a</sup>	35/67 <sup>ab</sup>	28/33 <sup>ab</sup>	101 <sup>ab</sup>	125/13 <sup>b</sup>
	Full bloom of white flowers	14/37 <sup>bc</sup>	24/33 <sup>b</sup>	18 <sup>bc</sup>	67/67 <sup>bc</sup>	109/77 <sup>C</sup>

Since the performance of thyme is different in different growth stages; Therefore, regarding the appropriate harvest times in order to increase yield in different climates, vegetative growth and flowering in different geographical areas are different depending on their weather conditions. Generally, thyme has the highest amount of essential oil during the flowering period, so this period is considered as the best time for harvesting (Stahl and Saez, 2002).

Most of the morphological indicators, including fresh and dry weight of the whole plant, height, large and small diameter, environment and dry yield of the plant per hectare showed the highest amount during flowering and in the agricultural ecosystem, except for fresh weight and leaf area, which were at the time of flowering and They showed the highest amount in the natural ecosystem. This is due to the fact that the plants in the agricultural ecosystem grow more due to more handling and care and more appropriate irrigation, and as a result, morphological indicators are more in the agricultural ecosystems.

By investigating the effect of altitude on the quantity and quality of the essential oil of mountain thyme in Damavand region, the results showed that the medicinal value of thyme depends on its essential oil, therefore, in high areas and natural ecosystem conditions, the best place to grow this plant is to obtain the best performance of the quantity and quality of the essential oil of the plant. (Jamshidi et al. 2015). Harvesting time in the three stages of the beginning of flowering, full flowering and after full flowering was evaluated on the amount and volume of garden thyme essential oil in Turkey. The results showed that harvesting time has a significant effect on plant height, fresh and dry weight, and essential oil content. The maximum essential oil was obtained when the harvest was done at the beginning of flowering (Kizil, 2006). By examining the study of Ozguven et al. (1998), the effect of environmental conditions and different stages of growth on the product and essential oil of garden thyme was investigated. In this research, with the essential oil extracted from the leaves, it was found that the environmental conditions and the age of the plant significantly affect the performance of the plant, so that the amount of essential oil obtained is from 0.32% to 0.83%. The percentage was variable.

The results of analysis of variance showed a significant difference between the interaction effect of harvest time and ecosystem type with the amount of chlorophyll a, b, a/b and T at the statistical level of 1%. The mentioned results are reported in Table 1-4 based on degrees of freedom and mean square. By comparing the investigation level of two agricultural and natural ecosystems, as well as the time of harvesting, the amount of chlorophyll of the plant was investigated (Table 6). As it is clear in table 7, the color of thyme flower is effective on the amount of chlorophyll of the plant. The highest chlorophyll a was observed in the natural ecosystem and at the time of full flowering of white flower at the rate of 32.08 mg/gm of dry weight and the lowest at the time of full flowering of purple flower at the rate of 15.88 mg/gm of dry weight. The highest amount of chlorophyll b in the natural ecosystem and before flowering is 22.35 mg per dry weight and the lowest is related to the agricultural ecosystem and before flowering with 9.72 mg per gram of dry weight. The results of chlorophyll a/b also showed that both in the time before flowering, the highest amount is related to the agricultural ecosystem at the rate of 1.85 mg/g of dry weight and the lowest in the natural ecosystem with 0.81 mg/g. It is dry weight. According to the results of total chlorophylls, the highest amount of chlorophylls in the place of the natural ecosystem and at the time of full flowering of white flower is 50.08 mg/g dry weight, and in the place of the agricultural ecosystem and the time before flowering, it is 65 27 mg/g of dry weight was the lowest amount of chlorophyll.



Table 6. Variance analysis of the effect of different ecosystems and different harvest times on the biochemical parameters of mountain thyme

Sources of Variation	df	Chlorophyll a	chlorophyll b	total chlorophyll	carotenoid	anthocyanin	flavonoid	phenol
place	1	ns23/83	95/66 *	ns0/315	0/026 **	ns2/081	6/04 **	ns 0/014
error	4	3/09	10/51	0/052	7/04	2/73	0/055	0/056
harvest time	2	137/59 **	ns10/44	ns0/19	ns14/60	91/24 **	1/31 **	ns 0/016
Interaction place*time	2	117/41 **	113 **	0/68**	ns13/92	23/85 **	ns0/52	ns 0/008
error	8	4/29	20/38	0/065	15/86	1/57	0/14	0/075

Table 7. Comparing the average effects of harvesting time and type of ecosystem on the biochemical parameters of mountain thyme

Check level	harvest time	Chlorophyll a	chlorophyll b	chlorophyll a/b	chlorophyll T
Agricultural	before flowering	<sup>b</sup> 17/93	<sup>b</sup> 9/72	<sup>a</sup> 1/85	<sup>d</sup> 27/65
	Full bloom of purple flowers	<sup>b</sup> 20/05	<sup>ab</sup> 15/95	<sup>b</sup> 1/3	<sup>bc</sup> 36/00
	Full bloom of white flowers	<sup>b</sup> 19/70	<sup>b</sup> 12/90	<sup>ab</sup> 1/59	<sup>c</sup> 32/60
Natural	before flowering	<sup>b</sup> 16/62	<sup>a</sup> 22/35	<sup>c</sup> 0/81	<sup>ab</sup> 38/97
	Full bloom of purple flowers	<sup>b</sup> 15/88	<sup>b</sup> 11/34	<sup>ab</sup> 1/41	<sup>d</sup> 27/22
	Full bloom of white flowers	<sup>a</sup> 32/08	<sup>ab</sup> 18/72	<sup>ab</sup> 1/72	<sup>a</sup> 50/08

According to the results of analysis of variance and F test, a significant difference between the type of ecosystem in the three parameters of anthocyanins, carotenoids and flavonoids was determined at the 1% statistical level ( $P < 0.01$ ). Therefore, according to Table No. 8, the highest amount of anthocyanins and flavonoids in the natural ecosystem is 19.22 and 3.66 mg/g dry weight, respectively, and carotenoids did not change much in both ecosystems. Both the type of ecosystem and the harvesting time were effective, so that the plants that were harvested in the stage before flowering and from the natural ecosystem had more flavonoids, anthocyanins and carotenoids than other treatments.

Table 8. Comparison of the average effect of different ecosystems on the biochemical parameters of mountain thyme

Check level	Anthocyanin)mg/gr(	carotenoid)mg/gr(	flavonoid)mg/gr(
Agricultural	<sup>b</sup> 18/54	<sup>a</sup> 12/03	<sup>b</sup> 2/51
Natural	<sup>a</sup> 19/22	<sup>a</sup> 12/11	<sup>a</sup> 3/66

According to the results of the present research, it can be stated that due to the favorable weather conditions that exist in the natural ecosystems and habitats of mountain thyme medicinal plants, most of the biochemical substances, including the amount of chlorophyll a, b and T, carotenoids, anthocyanin and flavonoid increases and only the amount of chlorophyll a/b showed the highest amount in the agricultural ecosystem. Regarding the time of harvesting, the amount of chlorophyll a, chlorophyll T and anthocyanin at the time of

harvesting the full flowering of white flower plants was higher than the previous flowering stage. This is while the amount of chlorophyll b, chlorophyll a/b and flavonoid was the opposite and had the highest amount before flowering.

he results of analysis of variance showed a significant difference between different harvest times and the interaction effect of harvest time and ecosystem type with the amount of essential oil at a statistical level of 1%, in other words, the interaction effect of harvest time and ecosystem type had a very significant effect on the amount of essential oil. (Table 9). Since the essential oil samples for analysis by gas chromatographic (GC) machine are mixed with each other and measured with the amount of essential oil constituents in different treatments and the main compounds of mountain thyme essential oil (compounds more than 3%) include: thymol, carvacrol, linalool, para-cymene, alpha-pinene and alpha-terpinene were identified and their amount was determined in the essential oil (Hornok, 1992; Furia and Bellanca, 1995; Goodner et al., 2006).

The quantity and quality of essential oils in plants are significantly influenced by various internal factors such as sex, genetics, seasonal changes and diversity related to plant growth and external factors in the form of ecological and environmental aspects such as altitude, length and latitude. Geography, soil, climate, light, etc. are mentioned, so the quantity and quality of essential oil compounds are different in different conditions (Stahl and Saez, 2002). Nik Avar et al. (2001) investigated the constituents of the essential oil of the flowering branches of *Thymus daenensis* in the Faculty of Pharmacy of Tehran University of Medical Sciences and the results showed that *Thymus daenensis* essential oil contains 26 compounds equivalent to approximately 99.7%, which are the main components of the essential oil. They included thymol 74.7%, paracymen 6.5%, beta caryophyllene 3.8% and methylcarvacrol 3.6%.

Nik Avar et al. (2001) investigated the constituents of the essential oil of the flowering branches of *Thymus daenensis* in the Faculty of Pharmacy of Tehran University of Medical Sciences and the results showed that *Thymus daenensis* essential oil contains 26 compounds equivalent to approximately 99.7%, which are the main components of the essential oil. They included thymol 74.7%, paracymen 6.5%, beta caryophyllene 3.8% and methylcarvacrol 3.6%.

The highest amount of para-cemen (16.2%) was at the time of full flowering of purple flower plants in the natural ecosystem and the lowest amount of para-ceemen (7.8%) was at the time of harvesting before flowering in the agricultural ecosystem. The highest amount of alpha-pinene (19.8%) was at the time of harvesting before flowering in the natural ecosystem and the lowest amount of alpha-pinene (7.8%) was at the time of harvesting the full flowering of purple flower plants in the agricultural ecosystem. The highest amount of alpha-terpinene (12.9%) was at the time of harvesting before flowering in the natural ecosystem, and the lowest amount of alpha-terpinene (8.9%) was at the time of harvesting the purple flower plants in the agricultural ecosystem.

The amount of essential oil in the cultivated ecosystem was higher than that of the natural ecosystem, and full flowering also resulted in an increase in the amount of essential oil, and the plants whose flower color was purple had a higher percentage of essential oil. But the essential oil compounds were more in the natural ecosystem and the essential oil compounds were different at different harvest times. The amount of thymol at the time before flowering and carvacrol at the time of flowering in plants with purple flowers and linalool at the time of flowering in plants with white flowers and para-simene at the time of flowering in plants with purple flowers and alpha-pinene at the time of flowering in plants with white flowers and alpha-pinene Terpinen showed the highest amount before flowering. The ratio of leaves to stems and the amount of essential oil are factors that affect the quality of the product. The

higher the number of leaves, the better the quality of the plant. In order to obtain more than 50% of leaves, harvesting should be done before flowering, and to obtain the highest amount of essential oil, harvesting during flowering is preferable (Stahl and Saez, 2002).

Table 9. Variance analysis of the effect of different ecosystems and different harvest times on the amount of mountain thyme essential oil

Sources of Variation	DF	Square Scale %essential oil
place	1	<sup>ns</sup> 0/75
error	4	0/1
harvest time	2	<sup>**</sup> 0/8
Interaction plase*time	2	<sup>**</sup> 0/42
error	8	0/02

Table 10. The amount of major components of mountain thyme essential oil

place	harvest time	Thymol%	%carvacrol	% linalool	%para- cymene	%alpha- pinene	alpha- %terpinene
Agricultural	before flowering	31/2	13/3	3/5	7/8	14/8	10/9
	Full bloom of purple flowers	13/3	18/2	9/4	12/8	7/8	8/9
	Full bloom of white flowers	16/8	14/3	7/6	9/5	10/4	9/8
Natural	before flowering	34/2	14/4	7/7	10/3	19/8	12/5
	Full bloom of purple flowers	26/7	19/4	12/1	16/2	13/5	11/8
	Full bloom of white flowers	27/5	15/4	13/2	14/8	15/9	11/7

Also, in the case of thyme essential oil, it had the highest percentage of essential oil at the time of full flowering and the agricultural ecosystem, but the quality and amount of essential oil compounds were observed to be higher in the natural ecosystem than in the agricultural ecosystem. Based on the studies of Goodner et al. in 2006, he also stated that agricultural factors have a great effect on the amount of essential oil and environmental conditions such as weather conditions, water and nutrition during harvest and after harvest, storage and processing conditions affect the amount of the product. For this reason, it is necessary to determine the optimal level of agricultural factors effective on plant growth and production.

## Conclusion

Therefore, according to the results obtained from the project, it can be said that by growing thyme plant in agricultural land, better yield is obtained in terms of dry weight of the plant and more amount of essential oil. This factor has a positive effect on economic factors, job creation in the agricultural and industrial sectors, and contributes to the progress of the society. However, due to the low density and large distances in the natural ecosystem, the dry weight yield of the plant is very low and harvesting is not economical, but in the natural ecosystem, the quantity and quality of thyme essential oil is definitely much better.

## References

- Amvam, P.H., Biyiti, L., Tchoumboungang, F., Menut, C., Lamaty, G. and Bouchet, Ph.,1998, Aromatic plants of tropical central Africa, Part XXXII, chemical composition and antifungal activity of thirteen essences from aromatic plants of Cameroon. *Flavour and Fragrance Journal*; 13: 107-114.
- Askari, F., Sefidkon, F., Allahverdi- Mamaghani, B., 2023. Efficiency of medicinal and aromatic plant's Essential oil for producing antimicrobia products. *Iran's nature*. p38.N1.57-71.
- Balbaa, S.I. 1983. Satisfying the requirements of medicinal plant cultivation. *Acta Horticulture*, 132: 73-84.
- Baser, K.H.C.,1999, Industrial utilization of medicinal and aromaticplants, *Acta Horticulturae*, 503: 177-192.
- Bentley, R. and Trimen, H.,1991, *Medicinal Plants*, Vol.3, Jowhar Offset Press, India, No. 205.
- Bernath, J. (1986). Production on ecology of secondary plants products, Herbs, spices and medicinal plants. Vol.I. Oryxpress, Arizona, 185-234.
- Brantner, A., Kartnig, T., Quehenberger, F. (1994). Comparative phytochemical investigation of *Hypericum perforatum* L. and *Hypericum maculatum* Crantz. *Scientia Pharmaceutica*,62:261-276.
- Briese, D.T. (1985). A survey to evaluate the longterm relationship between *Chrysolina quadrigemina* and its host-weed *St. John's word* in southeastern Astraulia. 6<sup>th</sup> International Symposium on Biological Control of Weed, pp.691-708.
- Brown, R.G., (2002). *Dictionary of medical plants*, Sarup and Sons, Publishers, Dehli, India.
- Carrapiso, A., Rubio, A., Sánchez-Casas, J., Martín, L., Martínez-Cañas, M., Miguel, C.,2020,Effect of the Organic Production and the Harvesting Method on the Chemical Quality and the Volatile Compounds of Virgin Olive Oil over the Harvesting Season.*MDPI, Food*,9(12), 1766.
- Ceylan, A., Bayram, E. and Ozay, N.,1994, The effects of N- fertilizer on the yield and quality of *Thymus vulgaris* in ecological condition of Bornova- Ismir, *Turkish Journal of Agriculture and Forestry*, 18: 249-255.
- Elahian., F, Garshasbi., M.,Mehri Asiabar. Z., Gholamian Dehkordi., N.,Yazdinezhad A., Mirzaei., A., 2021, Ecotypic Variations Affected the Biological Effectiveness of *Thymus daenensis* Celak Essential Oil,Hindawi Evidence-Based Complementary and Alternative Medicine, Article ID 6686558, 12 pages <https://doi.org/10.1155/2021/6686558>
- Cosson, L. (1966). Influence De eclairement sur teneur en alcaloides des Daturas. *Herba Hungarica*,5:157e.
- Fabio, A., Cermelli, C., Fabio, G., Nicoletti, P. and Quaglio, P.,2007, Screening of the antibacterial effects of a variety of essence on microorganisms responsible for respiratory infections, *Phytotherapy-Research*; 21(4): 374-377.
- Flodesi, D., Svab, J., Doby, M. (1968). Experimental results on advanced methods of the larg svale cultivation of sweet Fennal in Hungary. *Novenytermeles*, 1:59-68.
- Fluck, H. (1955). The influence of climatic on the active principles in medicinal plant. *Journal of Pharm*, 7: 361-383.
- Franz, Ch. (1983). Nutfrient and water management for medicinal and plants. *Acta Horticulture*, 188:21-27.
- Furia, T. E. And Bellanca, N.,1995, *Fenarolis Hand book of Ingredients*, Vol. 1, 3rd Edition CRC Press: 256, 272, 273.
- Furia, T. E. And Bellanca, N.,1995, *Fenarolis Hand book of Ingredients*, Vol. 1, 3rd Edition CRCPress: 256, 272, 273.
- Galambosi, B. (1993). Consideration and experience regarding the cultivation of medicinal wildflowers in Finland. *Aquilo Ser Botanica*,31:161-166.
- Gao, X., Ohlander, M., Jeppsson, N., Bjork, L. and Trajkovski, V. (2000) Changes inantioxidant effects and their relationship to phytonutrients in fruit of sea buckthourn (*Hippophae rhamnoides*. L) during maturation. *Journal of Agriculture and Food Chemistry* 48: 1458-1490.
- Goodner, K.L., Mahattanatawee, K., Plotto, A. and Sotomayor, J.A.,2006, Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essence by GC-MS/GC-O., *Jordan, M-J Industrial Crops and Products*; 24(3): 264-268.

- Goodner, K.L., Mahattanatawee, K., Plotto, A. and Sotomayor, J.A., 2006, Aromatic profiles of *Thymus hyemalis* and Spanish *T. vulgaris* essential oils by GC-MS/GC-O., Jordan, M-J Industrial Crops and Products; 24(3): 264-268.
- Grunwald, J., Graubau, H.J., Busch, R., Bentley, C. and Fiebich, B., 2006, *Thymus kotschyanus* and primrose root: a powerful synergism for the therapy of acute bronchitis, Zeitschrift für Phytotherapie; 27(5): 214-220.
- Golparvar, A., Hadipanah, A., 2014. Comparative Effect of Harvest Time on Essential Oil and Thymol Content of (*Thymus vulgaris* L.) and (*Thymus daenensis* Celak) in Iran Province. Electronic Journal of Biology, 2014. Vol. 10(3): 85-92.
- Hornok, L. (1978). Gyógynövényk termesztése és feldolgozása mezőgazdasági. Kiadó, Budapest.
- Hornok, L., 1992, Cultivation and processing of medicinal plants, Akademia Kiadó. Budapest, Hungary: 200-205.
- Hornok, L., 1992, Cultivation and processing of medicinal plants, Akademia Kiadó. Budapest, Hungary: 200-205.
- Hornok, L., Foldesi, D. and Szasz, E., 1975, Experiments of adapting the growing methods of common *Thymus kotschyanus* (*Thymus vulgaris*), Herba- Hungarica, 14: 47-64.
- Iftikhar, T., Majeed, H., Shehwar Zahra, S., Waheed, M., Niaz, M. Bano, N., 2023., Thyme., Essentials of Medicinal and Aromatic Crops pp 399–429.
- Kartnig, T., Heydel, B. (1993). Effects of visible and ultraviolet lights on the production of hypericin and flavonoids in cell cultures of *Hypericum perforatum*. Planta Medica, 59:654.
- Kerekes, J. (1969). Medicinal producing. Mezogaz asagi kiado, Budapest.
- Krizek, D.T., Kramer G.F., Upadhyaya A. and Mirecki R.M. 1993. UV-B Response of cucumber seedling grown under metal halid and high pressure sodium/deluxe lamps. Physiology of Plant. 88:350-358.
- Krol, B., Kiełtyka-Dadasiewicz, A., 2015, Yield and Herb Quality of Thyme (*Thymus Vulgaris* L.)
- Lichtenthaler HK (1987) Chlorophyll and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148:350-382.
- Meyer, F. (1973). Der Einfluss des Gebirgsklimas auf die cardenolid glykoside von *Digitalis lanata* Ehrh., university Doctoral Dissertation, Zurich.
- Morgan, R.K., 1989, Chemotypic characteristics of *Thymus vulgaris* L. in Central Otago, New Zealand. J. Biogeography; 16: 483-491.
- Mossa, J.S., Al-Yahya, M.A. and Hassan, M.M.A., 1987, Physicochemical characteristics and spectroscopy of the volatile oil of *Thymus vulgaris* growing in Saudi Arabia, Int. J. Crude Medicine Res; 25: 26-34
- Omidbaigi, R., Hornok, L. (1992) Effect of N fertilization on the production of fennel (*Foeniculum vulgare* Mill). Acta Horticulture, 306:249-254.
- Ozcan, M. and Chalchat, J.C., 2004, Aroma profile of *Thymus vulgaris* L. growing wild in Turkey. Bulgarian-Journal-of-Plant-Physiology; 30(3/4): 68-73
- Piccalgia, R., and Morotti, M., 1994. Characterization of several aromatic plants growing in northern Italy, Horticultural Abstracts, 64: 1368.
- Picuric- Jonanovic, K., Milovanovic, M. and Vrbaski, Z., 1995, *Thymus vulgaris* as a source of natural antioxidants: Review of Research- Work at the Faculty of Agriculture- Belgrade, USSR, 40: 141-146.
- Prakash, V., 1990, Leafy Spices, CRC Press, USA: 99-102.
- Proter, NG., Lammerink, J.P., 1994, Effect of temperature on the relative densities of essence and water, Journal of Essential Oil Research; 6: 3, 269-277.
- Rada, F., Gonzalez, J., Azocar, A., Briceno, B., Jaimez, R. (1992). Net photosynthesis leaf temperature relation in plant species with different height and altitudinal gradient. Acta Oecologica, 13:535-542.

- Saez, F.,1995, Essential oil variability of *Thymus hyemalis* growing wild in southeastern Spain. Biochem. Syst. Ecol; 23: 431- 438.
- Sliwinska, A., Bazylo, A. and Strzelecka, H.,2001, Spasmolytic effect of herbal *Thymus kotschyanus* and its extract, Herba-Polonica; 47(1): 56-69.
- Southwell, J.A., Campbell, M.H. (1991). Hypericin content variation in *Hypericum perforatum* in Australia. Phytochemistry,30:475-478.
- Stahl-Biskup, E. and Saez, F., 2002, Thyme; The genus *Thymus*, Taylor & Francis publishers, London and New York.
- Stahl- Biskup, E. and Saez, F., 2002, *Thymus kotschyanus*; The genus *Thymus*, Taylor & Francis publishers, London and New York.
- Storanova, M., Apostolova, B. (1992). The influence of altitude upon the biochemical composition of tutsan (*Hypericum perforatum*). Naukazagorata, 29:7-82.
- Titz, A., 2004. Medicinal herbs and plants scope for diversified and sustainable extraction, July, Bangalore. Pakistan/India.
- Tivy, J. (1993). Agricultural Ecology. Longman Scientific & Technical. 288p.
- Wills, R.B.H. Bone, K. and Morgan, M., 2000, Herbal products: active constituents, modes of action and quality control, Nutrition Research Reviews, 13: 47-77.
- Yamaura, T., Tanaka, S., Tabata, M., 1992 Localization of biosynthesis and accumulation of monoterpenoids in glandular trichomes of *Thymus kotschyanus*, Planta Medica; 58:153-158