

# Soxhlet Extraction of Cannabinoids (CBD and THC) From Four Different Strains of Cannabis Grown in Four Different Regions

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## Research Article

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# Abstract

The objective of this work was to investigate the cannabinoid content of extracts which was conducted by soxhlet extraction. In the extraction system hexane, ethyl acetate and ethanol were used sequentially to extract cannabis plant. When all species were evaluated in terms of CBD, all results in Meram region were the highest. As it can be understood, if an efficient extract in terms of CBD is desired, sowing can be done in Meram. Narlı strain provided the highest (65.88%) yield in terms of CBD in accordance with its cultivation purpose. When evaluated THC active ingredient, it was seen that each strain gave high yields in different regions. Elnur strain yielded the highest yields in Altinekin (66.21%), Papatya in Beyşehir (52.05%), Narlı (14.49%) and Gökçe ağaç in Çumra (56.13%). It is the first study to show the different climatic conditions effect on the yield of CBD and THC in different strains.

## 1. Introduction

Until early 1940, *Cannabis sativa* L. was widely cultivated in Turkey for production of hemp and hemp-derived products, mostly employed for industrial purposes. Hemp fibres were largely used in textiles, paper and ropes because of their strength and durability, but they were mostly replaced by synthetic ones over the years. Production decreased from year to year to 10 deceres in 2015. One of the important reasons for this decline was the privatization of the factory which uses hemp as a raw material in 2004. The privatized factory purchases the hemp need from abroad because it is cheaper, bringing the hemp production in Turkey to the end [1]. Cannabis was brought back to the agenda as an agricultural product at the "Symposium on Local Governments in the Presidential Government System" held on January 9, 2019. The purpose of Turkey's recent hemp policy is to produce and spread hemp production to meet domestic needs.

Cannabis (*Cannabis sativa* L.) is a herbaceous plant that has found commercial value in textile, food, papermaking and construction industry for centuries. Several countries, such as Australia, France and the United States, have recently permitted cultivation of low (<0.3% w/w) tetrahydrocannabinol (THC) hemp [2]. The plants of *Cannabis sativa* L. produce more than 100 natural cannabinoids, known as phytocannabinoids [3] divided in psychotropic and non-psychotropic cannabinoids. In the fresh plant material, most of the cannabinoids are initially synthesized as non-psychotropic carboxylic acids. These precursors are converted into the neutral form after decarboxylation induced by drying, heating, combustion or aging [4]. While there is no male and female distinction in cannabis seeds, there is a distinction between male and female in grown plants. It is known that there are less cannabinoids in male plants than in female [5].

Increasing evidences suggested that cannabinoids can be used in different pathological conditions, such as symptoms of muscle problems associated to multiple sclerosis, nausea and vomiting caused by chemotherapy, and anorexia in cancer or HIV patients [6, 7].

The main cannabinoids are  $\Delta^9$ -Tetrahydrocannabinol (THC) and its structurally analogue, although not psychoactive, cannabidiol (CBD). They are present in the plant as inactive acids (THC-A and CBD-A) and they can be converted in the corresponding non-acidic forms via thermal decarboxylation [8].

As the analysis of cannabis has gained new global importance, mainly for quality control within the legalized recreational and medical cannabis industry, and also for forensic differentiation between drug-type cannabis and legal products such as fibre hemp and CBD-rich and THC-poor cannabis the importance of the correct choice of an extraction method for the extraction of cannabinoids from various matrices e.g. cannabis plant extracts, hemp food products, biomass, cannabis oils, whole blood, plasma, oral fluids, hair and so on, has become paramount [9, 10]. There are a wide variety of cannabinoids in cannabis. The occurrence of these cannabinoids in different parts of the cannabis is strongly dependent on the genetic expression and growing environmental conditions. Such as, female plants gives higher quantity of cannabinoids at the stage of maturity. While, male plants are preferable for higher yields of the non-cannabinoids and flavonoids i.e. hemp form [11].

The choice of an extraction method relies on the nature of the source material, e.g. dried plant powder, biological materials, soil or water, as well as the target compounds, e.g. cannabinoids [12]. Prior to deciding on a particular extraction method for naturally occurring cannabinoids, one must consider the following: purpose of extraction, quantity of extraction, purification steps to be carried out, purity level of cannabinoids, possible artefact formation, stability of target cannabinoids, physicochemical properties of target cannabinoids, and obviously the cost and environmental impacts. In addition to traditional methods like maceration, distillation or boiling, several other modern extraction methods and techniques can be applied for the extraction of naturally occurring cannabinoids, and those methods include, soxhlet, accelerated solvent extraction, pressurized liquid extraction (PLE), microwave-assisted extraction (MAE), ultrasound assisted extraction (UAE), supercritical fluid extraction (SFE), solid phase extraction (SPE) and micro solid-phase extraction (MSPE) [12, 13]. The choice of solvent for extraction is equally important to have maximum extraction yield. For the extraction of naturally occurring cannabinoids, most often, organic solvents are preferred, because of the lipophilic nature of most of the naturally occurring cannabinoids.

Traditional methods, like maceration, percolation, and soxhlet, are known to have some limits such as time and solvent consumption, and decomposition of heat sensitivity bioactive compounds [14]. However, soxhlet technique is still common in laboratories and industries being involved in a wide variety of official methods [15].

According to the scanning results of the literature, generally hemp seed, seed oil yield, essential oil composition or mostly non-psychoactive ingredient CBD and sometimes only THC have been researched. And also there are a few researches by ultrasonic and microwave extractions. Apart from extraction methods, various analytical methods (such as GC-MS and HPLC) have been reported for the analysis of cannabinoids in cannabis plant. There was no publication in the literature that cultivated several strains at the same time, developed a soxhlet extraction procedure, and analyzed CBD and THC at the same time.

Searching “cannabidiol” in literature scanning web-sites and filtering results just for the last 20 years, it is amazing to realize that most of the work, about 60%, are interested on pharmacological activity of the compound, 30% are still researching and progressive analytical methods able to separate the presence of other cannabinoids. Finally, only 6% of the published researches is interested in the extraction protocols.

Therefore in this context, the aim of the present study was to develop and a soxhlet extraction procedure to get CBD and THC extracts from the different four strains which planted from different four regions of Konya, Turkey. It is the first study to show the different climatic conditions effect on the yield of CBD and THC.

## 2. Materials And Methods

### 2.1 Chemicals

Soxhlet extraction solvents which are hexane, ethyl acetate and ethanol were purchased from Sigma, Germany. Cannabinoid standards (CBD and THC) were supplied at 1 mg/mL stock solutions in methanol by Sigma, Germany, Acetonitrile (Sigma, Germany), water (Ultrapure for chromatographic analysis), Acetic acid (100% anhydrous, glacial, Merck) and methanol (Sigma, Germany).

### 2.2 Seeds

Four different cannabis seeds were purchased from local markets in Turkey. Two of them from Konya (Elnur and Papatya) and one of them from Ankara (Gökçeağaç) and the last one is “Narlı” from Narlısaray district from Samsun. Only the Narlı is genus, the others are the population. Narlı, the first domestic and national seed variety, was officially registered at the end of 2019 with the work carried out within the scope of the "Development of Genotypes with Low THC in Cannabis Populations Project" initiated by Samsun Ondokuz Mayıs University Faculty of Agriculture in Turkey. The purpose of developing this variety is to produce hemp with low THC and to transform it into products such as fiber.

### 2.3 Cultivation Regions

The seeds were planted at four different regions which have different climates in Konya, Turkey. You can see the characteristics of the planted regions in supplementary material (**Table S1**). In the table (**S1**), longitude, latitude, altitude (m), annual rainfall (mm), average temperature (°C), average annual relative humidity (%) values were indicated. All the regions are differ from each other in terms of indicated climate values. Planting was carried out on the dates specified in Altınekin (13 June 2019), Çumra (18 June 2019), Meram (18 June 2019), and Beyşehir (14 June 2019) districts of Konya.

### 2.4 Harvesting, drying, storing and picking

In the cannabis plant, male and female plants mature at different times from each other. Male plants mature faster than female plants. Considering this difference, there are different harvesting methods in our country. We harvested both at the same time, keeping the male plants waiting until the female plants were mature.

The harvested plants were kept in the greenhouse without direct sunlight, according to the regions, so that the male and female did not interfere. The plants were then hand-picked without using a mechanical tool so that the leaves were separated from their stems. The drying process will continue in the oven at 30°C for 48 hours (low temperature and for a long time) until the samples reach the grinding conditions.

## 2.5 Sample preparation

The leaves were splintered at 3 degrees speed for 3 minutes using Waring Commercial Laboratory Blender (Sigma, Germany). Every strain was weighed 100.0 g portions and stored at -18°C until to use.

### 2.5.1 Decarboxylation process

The plants were exposed to the decarboxylation process at 140°C for 30 min before extraction according to the Grijo et al. [5].

## 2.6 Soxhlet extraction procedure

The CBD and THC present in the raw material was extracted using the Soxhlet method. The extractions were performed under reduced pressure to avoid the thermal degradation of the target compound. The extractions were carried out in a six-hour period, using a solvent polarity gradient - first hexane, then ethyl acetate, and finally ethanol. Briefly, 10.0 g of cannabis plant material was extracted with hexane, obtaining the non-polarity fraction. Subsequently, the residual cake was extracted with ethyl acetate, producing the middle-polarity fraction. Finally, the next residual cake was extracted with ethanol, thus getting the polar fraction. All fractions were concentrated under vacuum and analyzed by HPLC.

The extraction yields were calculated according to the equation:

$$\text{Yield (\% w/w)} = \text{mass dried extract (g)} / \text{mass dried matrix (g)} \times 100 \text{ Eq-1}$$

The results were expressed as the average of two replicates of the extraction.

## 2.7 Determination of CBD and THC with HPLC

Cannabinoid composition was analyzed with HPLC (Waters e2695) equipped with a ACE- 5 C18 250×4,6 mm id 5µm column at 30°C oven temperature. Mobile phase solvents were acetonitrile with 0.1% (v/v) acetic acid and water were run at isocratic system. Analysis were run at 210 nm. Injection volume was 20 µL. Flow rate was 1 mL/min. Run time was 65 min. HPLC system has a 2489 UV/vis detector. This conditions were selected based on the literature with some modifications. Concentrations of CBD and THC were quantified by their peak areas against those of standards. All the samples concentrations were 1 mg/1 mL in methanol then diluted 50 times. Concentration of the standards were 12.5 ppm CBD and 10 ppm THC.

## 3. Results And Discussion

## 3.1 Extract yields

Choosing the right cannabis soxhlet extraction solvent can be complicated. Prices can vary widely, different processes require different levels of expertise to operate, and not all ground facilities will be suitable to handle some of the more hazardous solvents that are seen in select extraction systems. The cannabinoids are non-polar compounds, and so can be removed effectively from the cannabis plant using other non-polar substances. But some terpenes, a class of compound responsible for much of cannabis' flavor and aroma, are polar compounds.

The extract yields obtained for the four strains grown in four different regions are reported in Table 1. As you can see from the table, all yields of different strains (Elnur, Gökçe ağaç, Narlı, Papatya) and their genders comparisons and soxhlet solvent comparisons according to the different cultivation regions are reported.

Table 1  
Extract yields\* (%) of the all strains which grown in different regions.

Gender / Strain	Soxhlet Solvent	Region			
		Çumra (%)	Altinekin (%)	Meram (%)	Beyşehir (%)
Female / Elnur	Hexane	4.35 ± 0.57	4.37 ± 0.17	7.05 ± 0.31	11.27 ± 0.77
	Ethyl Acetate	1.15 ± 0.37	0.99 ± 0.002	3.13 ± 0.12	1.21 ± 0.02
	Ethanol	8.11 ± 0.21	1.45 ± 0.04	3.76 ± 0.23	1.59 ± 0.03
Male / Elnur	Hexane	4.44 ± 0.04	3.34 ± 0.46	4.14 ± 0.11	4.55 ± 0.17
	Ethyl Acetate	1.55 ± 0.09	1.49 ± 0.06	8.00 ± 0.56	0.96 ± 0.002
	Ethanol	10.96 ± 0.61	5.36 ± 0.15	2.91 ± 0.12	1.38 ± 0.12
Female / Gökçe ağaç	Hexane	3.21 ± 0.02	8.12 ± 0.38	2.81 ± 0.13	8.42 ± 0.55
	Ethyl Acetate	0.71 ± 0.003	3.96 ± 0.21	1.87 ± 0.08	1.73 ± 0.05
	Ethanol	3.80 ± 0.03	8.52 ± 0.47	4.94 ± 0.22	9.32 ± 0.32
Male / Gökçe ağaç	Hexane	4.58 ± 0.01	13.83 ± 0.78	3.85 ± 0.18	6.11 ± 0.36
	Ethyl Acetate	1.50 ± 0.18	3.39 ± 0.10	1.38 ± 0.07	1.52 ± 0.10
	Ethanol	2.12 ± 0.14	3.18 ± 0.12	5.58 ± 0.20	7.67 ± 0.35
Female / Narlı	Hexane	6.74 ± 0.25	8.84 ± 0.23	5.48 ± 0.21	9.90 ± 0.42
	Ethyl Acetate	2.26 ± 0.11	1.57 ± 0.08	1.64 ± 0.02	2.33 ± 0.06
	Ethanol	7.13 ± 0.31	2.22 ± 0.13	3.68 ± 0.05	3.12 ± 0.13
Male / Narlı	Hexane	2.99 ± 0.14	2.67 ± 0.20	3.79 ± 0.13	5.18 ± 0.21
	Ethyl Acetate	1.64 ± 0.09	1.75 ± 0.11	1.40 ± 0.02	2.38 ± 0.18
	Ethanol	6.15 ± 0.12	3.32 ± 0.54	2.47 ± 0.05	8.10 ± 0.36
Female / Papatya	Hexane	2.47 ± 0.08	4.24 ± 0.59	2.60 ± 0.09	4.56 ± 0.55
	Ethyl Acetate	1.56 ± 0.11	1.53 ± 0.17	1.93 ± 0.12	1.11 ± 0.07
	Ethanol	4.79 ± 0.18	3.04 ± 0.34	3.85 ± 0.03	2.61 ± 0.03
Male / Papatya	Hexane	5.45 ± 0.23	3.59 ± 0.44	3.30 ± 0.05	5.03 ± 0.14
* % wt, d.b.=dry basis					
Extractions were done by duplicate and values are expressed as mean ± SD.					

		Region			
	Ethyl Acetate	1.03 ± 0.05	1.88 ± 0.25	1.47 ± 0.11	1.74 ± 0.09
	Ethanol	2.81 ± 0.07	8.10 ± 0.76	5.43 ± 0.19	2.00 ± 0.13
* % wt, d.b.=dry basis					
Extractions were done by duplicate and values are expressed as mean ± SD.					

Looking at the table in terms of differences between species, it is seen that the highest extract yields were obtained from Gökçeağaç (13.83% and 8.42%). Although the second highest yield (11.27%) is obtained when the female of the Elnur species grows in the Beyşehir region, the second highest yields are all regions of the female of the Narlı species (9.90%, 8.84%, 6.74%, and 5.48%) considering the high yields obtained from each region.

When looking at the table in general terms, it is seen that the highest yields were obtained with hexane solvent (13.83% Gökçeağaç strain in Altınekin region) and the lowest yields were obtained with ethanol solvent. This idea is disrupted by the higher yields of ethanol than hexane yields of all species in Çumra region. Although higher amounts of cannabinoids are obtained with ethanol, which is considered here, we will explain in the section that we examine the content that this idea is not correct. It is understood that other components other than cannabinoids dissolved in the plant with ethanol solvent also come at this stage.

According to the extract yields, soxhlet seems to be the suitable technique for the extraction of plants aerial parts. However, it should be pointed out that this extraction technique carried out at high temperature allows the co-extraction of the fibers. These contribute to the dry extract weight. While the species with high yields cannot be considered to have high content of cannabinoids, the cannabinoid content of Papatya and Elnur species with low yields should not be considered to be low. Because in soxhlet extraction, hexane, ethyl acetate, and ethanol solvents chosen to make a transition from non-polar to polar in terms of polarity will not only extract the cannabinoid species in the cannabis plant. If the cannabinoid content of the highest yield species is also high, then we can say that we have made a selective extraction.

Palmieri et al., (2020) [16] were found that the yields of hemp which conducted soxhlet extraction system for 6 hours, used ethanol for solvent was 10.00%. This result is fitting with our ethanol extraction result part.

The difference in extract yields can also be evaluated in terms of climatic differences. Differences in latitude, longitude, temperature, rainfall and humidity of the cultivated regions given in **Table S1** cause differences in the development and content of the species. When evaluated from this point of view, it is seen that the highest yields are obtained from the regions with the highest rainfall and the lowest temperature in Altınekin and Beyşehir regions.

## **3.2 Composition determination of extracts with HPLC**

According to the method described in section 2.7, the calibration charts were drawn by running the CBD and THC standards at different concentrations. According to the standard chromatograms, all extracts were quantified. Analyses were done by duplicate and values are expressed as mean  $\pm$  SD.

### **3.2.1 The same strains which grown in different regions**

Four different cannabis strains cultivated in four different regions were used throughout the study.

#### **3.2.1.1 Elnur strain**

In Table 2, we can see how the Elnur strain, the first of these plants, differs in content when planted in different regions. According to the data in the table; when interpreted in general, we can say that female strain contain higher amounts of CBD and THC than males. When evaluated in terms of the solvent used in the soxhlet system, hexane was the solvent with the highest CBD and THC contents as expected. It can be said that there is a linear decrease in ethyl acetate and ethanol results according to polarity ranking. As we said when evaluating the extract yields, it cannot be said that high CBD and THC are obtained when the contents of the species with high extract yield are considered. To be more precise, high extract yields do not necessarily mean a high selective extraction.

Table 2  
CBD and THC results of Elnur strain which grown in different regions.

Strain	Region	Gender	Solvent	CBD %	THC %
Elnur	Meram	Female	Hexane	52.11 ± 1.95	40.11 ± 2.22
Elnur	Meram	Male	Hexane	18.96 ± 1.21	36.33 ± 2.05
Elnur	Meram	Female	Ethyl Acetate	25.49 ± 1.57	20.37 ± 1.54
Elnur	Meram	Male	Ethyl Acetate	4.23 ± 0.23	7.07 ± 0.85
Elnur	Meram	Female	Ethanol	0.19 ± 0.05	1.91 ± 0.12
Elnur	Meram	Male	Ethanol	0.25 ± 0.12	2.07 ± 0.08
Elnur	Altinekin	Female	Hexane	30.01 ± 1.11	66.21 ± 3.14
Elnur	Altinekin	Male	Hexane	16.28 ± 1.02	16.09 ± 1.23
Elnur	Altinekin	Female	Ethyl Acetate	6.47 ± 0.35	7.50 ± 0.23
Elnur	Altinekin	Male	Ethyl Acetate	9.09 ± 0.85	7.94 ± 0.15
Elnur	Altinekin	Female	Ethanol	0.00 ± 0.00	1.94 ± 0.07
Elnur	Altinekin	Male	Ethanol	0.00 ± 0.00	1.89 ± 0.09
Elnur	Çumra	Female	Hexane	47.65 ± 2.15	46.15 ± 1.11
Elnur	Çumra	Male	Hexane	44.33 ± 1.86	34.93 ± 1.05
Elnur	Çumra	Female	Ethyl Acetate	18.41 ± 1.10	9.13 ± 0.53
Elnur	Çumra	Male	Ethyl Acetate	7.09 ± 0.88	4.48 ± 0.32
Elnur	Çumra	Female	Ethanol	4.51 ± 0.56	2.38 ± 0.13
Elnur	Çumra	Male	Ethanol	2.79 ± 0.25	2.16 ± 0.12
Elnur	Beyşehir	Female	Hexane	30.60 ± 1.74	46.68 ± 1.87
Elnur	Beyşehir	Male	Hexane	49.76 ± 2.12	40.46 ± 1.99
Elnur	Beyşehir	Female	Ethyl Acetate	19.83 ± 1.05	15.68 ± 1.23
Elnur	Beyşehir	Male	Ethyl Acetate	7.63 ± 0.88	6.18 ± 0.98
Elnur	Beyşehir	Female	Ethanol	6.89 ± 0.96	2.80 ± 0.87
Elnur	Beyşehir	Male	Ethanol	0.00 ± 0.00	2.19 ± 0.75
HPLC analyses were done by duplicate and values are expressed as mean ± SD.					

When the differentiation of content of Elnur strain according to different regions is examined from Figure 1A-B-C-D and **Figure S3-A**, the highest CBD yield in Meram region (52.11%); we see that the highest THC

yield is obtained in the Altınekin region (66.21%). These results were examined in terms of female species. Because the general trend is that the results of the female species are higher than the male species. When Figure 1-A-B-C-D is examined, it is seen that hexane, ethyl acetate and ethanol results are obtained by decreasing and female species generally contain higher CBD and THC results than males. Only in the extracts obtained with hexane in the Beyşehir region, the CBD and THC contents of the female and male strains are close to each other. More THC was obtained in the Elnur species than expected and allowed by legal limits.

The climatic differences of the Meram and Altınekin regions where the highest yields were obtained are given in **Table S1**. It is Meram, which receives the least rainfall and is one of the regions with the highest altitude, and Altınekin, one of the regions with the highest rainfall and the lowest altitude.

### **3.2.1.2 Papatya strain**

The results of the contents of Papatya, another of the species, are given in Table 3. It is seen that the highest CBD yield was obtained in the Meram region (47.39%) and the highest THC yield was obtained from the Beyşehir region (52.05%) (**Table S3-B**). The tendency of female species to have higher yields than male species also applies to this species (Figure 2-A-B-C-D). The yields of hexane, ethyl acetate and ethanol again show a decreasing trend. Their yields in the Çumra region are very low. THC percentages are higher than expected and legal limits allow. The cultivation of this species in the Meram region is suitable for CBD yields. Considering the climatic differences of the regions, it should be noted that the Meram region is the region with the highest temperature and the least rainfall.

Table 3  
CBD and THC results of Papatya strain which grown in different regions.

Strain	Region	Gender	Solvent	CBD %	THC %
Papatya	Meram	Female	Hexane	47.39 ± 1.78	40.64 ± 2.22
Papatya	Meram	Male	Hexane	35.19 ± 1.61	37.83 ± 2.32
Papatya	Meram	Female	Ethyl Acetate	41.32 ± 1.98	29.63 ± 1.88
Papatya	Meram	Male	Ethyl Acetate	9.96 ± 1.08	9.07 ± 0.85
Papatya	Meram	Female	Ethanol	0.00 ± 0.00	2.07 ± 0.11
Papatya	Meram	Male	Ethanol	0.05 ± 0.003	1.81 ± 0.13
Papatya	Altinekin	Female	Hexane	24.76 ± 0.15	24.26 ± 0.52
Papatya	Altinekin	Male	Hexane	23.70 ± 0.27	33.27 ± 1.42
Papatya	Altinekin	Female	Ethyl Acetate	9.45 ± 0.16	10.37 ± 1.14
Papatya	Altinekin	Male	Ethyl Acetate	3.52 ± 0.09	5.63 ± 0.98
Papatya	Altinekin	Female	Ethanol	0.00 ± 0.00	1.86 ± 0.44
Papatya	Altinekin	Male	Ethanol	0.12 ± 0.007	1.91 ± 0.79
Papatya	Çumra	Female	Hexane	11.01 ± 0.38	28.23 ± 1.55
Papatya	Çumra	Male	Hexane	0.56 ± 0.003	1.83 ± 1.36
Papatya	Çumra	Female	Ethyl Acetate	14.99 ± 1.22	10.53 ± 2.02
Papatya	Çumra	Male	Ethyl Acetate	5.28 ± 0.88	5.00 ± 0.93
Papatya	Çumra	Female	Ethanol	0.06 ± 0.002	1.81 ± 0.62
Papatya	Çumra	Male	Ethanol	0.06 ± 0.004	1.82 ± 0.56
Papatya	Beyşehir	Female	Hexane	32.47 ± 1.66	52.05 ± 2.41
Papatya	Beyşehir	Male	Hexane	25.76 ± 1.85	30.10 ± 2.03
Papatya	Beyşehir	Female	Ethyl Acetate	8.94 ± 1.11	11.65 ± 1.79
Papatya	Beyşehir	Male	Ethyl Acetate	11.28 ± 1.12	9.90 ± 1.13
Papatya	Beyşehir	Female	Ethanol	0.00 ± 0.00	1.91 ± 0.84
Papatya	Beyşehir	Male	Ethanol	0.13 ± 0.002	1.83 ± 0.91
HPLC analyses were done by duplicate and values are expressed as mean ± SD.					

Table 4  
CBD and THC results of Narlı strain which grown in different regions.

Strain	Region	Gender	Solvent	CBD %	THC %
Narlı	Meram	Female	Hexane	54.51 ± 3.20	14.49 ± 0.82
Narlı	Meram	Male	Hexane	64.79 ± 3.74	5.28 ± 0.19
Narlı	Meram	Female	Ethyl Acetate	30.21 ± 2.85	3.49 ± 0.18
Narlı	Meram	Male	Ethyl Acetate	6.54 ± 1.01	1.97 ± 0.09
Narlı	Meram	Female	Ethanol	0.08 ± 0.003	1.78 ± 0.11
Narlı	Meram	Male	Ethanol	0.08 ± 0.001	1.77 ± 0.24
Narlı	Altinekin	Female	Hexane	54.67 ± 2.89	7.88 ± 0.07
Narlı	Altinekin	Male	Hexane	59.38 ± 2.77	4.52 ± 0.11
Narlı	Altinekin	Female	Ethyl Acetate	13.76 ± 1.20	2.79 ± 0.05
Narlı	Altinekin	Male	Ethyl Acetate	0.00 ± 0.00	1.90 ± 0.03
Narlı	Altinekin	Female	Ethanol	0.50 ± 0.02	1.77 ± 0.13
Narlı	Altinekin	Male	Ethanol	5.50 ± 0.22	1.77 ± 0.10
Narlı	Çumra	Female	Hexane	61.53 ± 2.44	8.70 ± 0.56
Narlı	Çumra	Male	Hexane	34.70 ± 2.12	5.81 ± 0.45
Narlı	Çumra	Female	Ethyl Acetate	34.31 ± 1.86	6.68 ± 1.12
Narlı	Çumra	Male	Ethyl Acetate	0.00 ± 0.00	2.09 ± 0.78
Narlı	Çumra	Female	Ethanol	0.28 ± 0.02	1.79 ± 0.91
Narlı	Çumra	Male	Ethanol	0.00 ± 0.00	1.80 ± 0.83
Narlı	Beyşehir	Female	Hexane	65.88 ± 2.33	8.11 ± 0.98
Narlı	Beyşehir	Male	Hexane	40.22 ± 1.75	3.15 ± 0.86
Narlı	Beyşehir	Female	Ethyl Acetate	26.97 ± 1.08	2.99 ± 0.77
Narlı	Beyşehir	Male	Ethyl Acetate	8.08 ± 0.74	2.00 ± 0.84
Narlı	Beyşehir	Female	Ethanol	0.56 ± 0.08	1.80 ± 0.22
Narlı	Beyşehir	Male	Ethanol	0.05 ± 0.009	1.77 ± 0.33

HPLC analyses were done by duplicate and values are expressed as mean ± SD.

### 3.2.1.3 Narlı strain

Narlı, the first domestic and national cannabis strain as we said in section 2.2, is the most efficient strain in terms of CBD among all cultivated strains for the purpose of production and cultivation. Looking at Table 3, it is seen that the hexane results of female species are 65.88% in Beyşehir region, 61.53% in Çumra region, 54.67% in Altınekin region and 54.51% in Meram region. Even the idea that female species results were higher than male species did not fit for this species, and even male species yielded CBD yields as much as females. (CBD yield obtained with hexane solvent in Meram region is 64.79%. CBD yield obtained with hexane solvent in Altınekin region is 59.38%.) The first noticeable content is CBD when looking at Figure 3A-B-C-D. In fact, in line with the cultivation logic, THC content is considerably less than all other species. When **Figure S3-C** is examined, it is seen that there will be no problem in terms of CBD when it is desired to be grown in all regions, but it will be obtained with a yield of over 60 percent in Beyşehir and Çumra regions. When it is considered as an extract with a lot of CBD but low in terms of THC, it is seen that the Beyşehir region is the most suitable region for growing Narlı strain. It can be seen from **Table S1** that the Beyşehir region receives the most rainfall and is the coolest region. The Narlı strain is the first for Turkey, as it is among the species that are allowed to be produced in terms of legal limits. In order to increase production and avoid legal obligations, incentives should be provided for the production of species that do not exceed legal limits in terms of THC rich in CBD.

### 3.2.1.4 Gökçeaağaç strain

When we look at Table 5 to examine the CBD and THC results of Gökçeaağaç, it can be said that the highest CBD yield is in the Meram region (56.18%), followed by the female species obtained with hexane solvent in the Altınekin region (50.49%). In general, low contents were obtained from Çumra region (Figure 4A-B-C-D). It seems that this region is not suitable for cultivating this strain. In the Beyşehir and Altınekin regions, it is seen that the CBD results are almost close to the THC results (obtained with hexane in female strains). In the Beyşehir and Meram regions, it is seen that quite a lot of CBD and THC are produced in male species. If a production rich in CBD is desired, if a THC-rich production is desired in the Meram region, Çumra and Altınekin regions should be preferred (**Figure S3-D**). Although the production of THC-rich species is prohibited in most of the countries, it can be ensured that the production of THC can be produced in a controlled manner, since it is used as a drug active substance in the field of medicine and especially as an analgesic.

Table 5  
CBD and THC results of Gökçe ağaç strain which grown in different regions.

Strain	Region	Gender	Solvent	CBD %	THC %
Gökçe ağaç	Meram	Female	Hexane	56.18 ± 2.98	45.81 ± 3.14
Gökçe ağaç	Meram	Male	Hexane	39.25 ± 2.33	42.21 ± 2.77
Gökçe ağaç	Meram	Female	Ethyl Acetate	33.55 ± 2.41	23.75 ± 1.57
Gökçe ağaç	Meram	Male	Ethyl Acetate	4.39 ± 1.20	5.70 ± 0.25
Gökçe ağaç	Meram	Female	Ethanol	0.10 ± 0.03	1.85 ± 0.14
Gökçe ağaç	Meram	Male	Ethanol	0.15 ± 0.02	1.85 ± 0.19
Gökçe ağaç	Altınekin	Female	Hexane	50.49 ± 2.66	52.72 ± 2.58
Gökçe ağaç	Altınekin	Male	Hexane	26.54 ± 1.87	21.54 ± 1.65
Gökçe ağaç	Altınekin	Female	Ethyl Acetate	22.79 ± 1.10	7.32 ± 0.18
Gökçe ağaç	Altınekin	Male	Ethyl Acetate	2.45 ± 0.72	1.88 ± 0.75
Gökçe ağaç	Altınekin	Female	Ethanol	11.84 ± 1.25	1.83 ± 0.33
Gökçe ağaç	Altınekin	Male	Ethanol	0.00 ± 0.00	1.99 ± 0.83
Gökçe ağaç	Çumra	Female	Hexane	4.46 ± 0.89	56.13 ± 3.11
Gökçe ağaç	Çumra	Male	Hexane	1.49 ± 0.03	3.63 ± 0.12
Gökçe ağaç	Çumra	Female	Ethyl Acetate	11.51 ± 1.41	8.48 ± 0.07
Gökçe ağaç	Çumra	Male	Ethyl Acetate	10.48 ± 1.35	5.00 ± 0.55
Gökçe ağaç	Çumra	Female	Ethanol	0.16 ± 0.08	1.85 ± 0.06
Gökçe ağaç	Çumra	Male	Ethanol	0.32 ± 0.01	1.93 ± 0.43
Gökçe ağaç	Beyşehir	Female	Hexane	42.27 ± 2.41	36.78 ± 2.15
Gökçe ağaç	Beyşehir	Male	Hexane	44.36 ± 2.58	32.57 ± 2.72
Gökçe ağaç	Beyşehir	Female	Ethyl Acetate	4.98 ± 0.55	6.61 ± 1.32
Gökçe ağaç	Beyşehir	Male	Ethyl Acetate	5.06 ± 0.83	4.49 ± 1.16
Gökçe ağaç	Beyşehir	Female	Ethanol	0.00 ± 0.00	1.82 ± 0.42
Gökçe ağaç	Beyşehir	Male	Ethanol	0.16 ± 0.02	1.82 ± 0.73

HPLC analyses were done by duplicate and values are expressed as mean ± SD.

### 3.2.2 CBD and THC results of all strains in dry cannabis sample

The yield results of CBD and THC contents of all strains in the different regions in dry cannabis sample are shown in Table 6. In the Çumra region, the highest amount of CBD in the dry plant was obtained from Narlı strain with 4.15%. In Altınekin region, the highest amount of CBD in dry plant is again Narlı strain with 4.83%. In the Meram region, the highest amount of CBD in the dry plant is the Elnur strain with 3.67%, but also the Narlı type is still at a considerable amount with 2.97%. In the Beyşehir region, the highest amount of CBD in dry plant was obtained from Narlı strain with 6.52%. In line with the production logic of the Narlı strain, it is an expected result that the highest yields of CBD amounts in dry plants were obtained from the Narlı strain. After the Narlı strain, higher yields are Gökçe ağaç and Elnur strains, respectively, grown in Altınekin and Beyşehir regions, which are obtained with the hexane solvent extraction of the females.

Table 6  
CBD and THC results of all strains in dry cannabis sample

in dry cannabis sample (%)									
Gender / Strain	Soxhlet Solvent	Çumra		Altinekin		Meram		Beyşehir	
		Region		Region		Region		Region	
		CBD	THC	CBD	THC	CBD	THC	CBD	THC
Female / Elnur	Hexane	2.07	2.01	1.31	2.89	3.67	2.83	3.45	5.26
	Ethyl Acetate	0.21	0.11	0.06	0.07	0.80	0.64	0.24	0.19
	Ethanol	0.37	0.19	0.00	0.03	0.01	0.07	0.11	0.04
Male / Elnur	Hexane	1.97	1.55	0.54	0.54	0.78	1.50	2.26	1.84
	Ethyl Acetate	0.11	0.07	0.14	0.12	0.34	0.57	0.07	0.06
	Ethanol	0.31	0.24	0.00	0.10	0.01	0.06	0.00	0.03
Female / Gökçe ağaç	Hexane	0.14	1.80	4.10	4.28	1.58	1.29	3.56	3.10
	Ethyl Acetate	0.08	0.06	0.90	0.29	0.63	0.44	0.09	0.11
	Ethanol	0.01	0.07	1.01	0.16	0.005	0.09	0.00	0.17
Male / Gökçe ağaç	Hexane	0.07	0.17	3.67	2.98	1.51	1.63	2.71	1.99
	Ethyl Acetate	0.16	0.08	0.08	0.06	0.06	0.08	0.08	0.07
	Ethanol	0.01	0.04	0.00	0.06	0.01	0.10	0.01	0.14
Female / Narlı	Hexane	4.15	0.59	4.83	0.70	2.97	0.79	6.52	0.80
	Ethyl Acetate	0.78	0.15	0.22	0.04	0.50	0.06	0.63	0.07
	Ethanol	0.02	0.13	0.01	0.04	0.003	0.07	0.02	0.06
Male / Narlı	Hexane	1.04	0.17	1.59	0.12	2.46	0.20	2.08	0.16
	Ethyl Acetate	0.00	0.03	0.00	0.03	0.09	0.03	0.19	0.05
	Ethanol	0.00	0.11	0.18	0.06	0.002	0.04	0.004	0.14
Female / Papatya	Hexane	0.27	0.70	1.05	1.03	1.23	1.06	1.48	2.37
	Ethyl Acetate	0.23	0.16	0.14	0.16	0.80	0.57	0.10	0.13
	Ethanol	0.003	0.09	0.00	0.06	0.00	0.08	0.00	0.05
Male / Papatya	Hexane	0.03	0.10	0.85	1.19	1.16	1.25	1.30	1.51

Since the results are calculated from the average results of the extracts, there is no need for standart deviation ( $\pm$ SD) in this table.

		in dry cannabis sample (%)							
	Ethyl Acetate	0.05	0.05	0.07	0.11	0.15	0.13	0.20	0.17
	Ethanol	0.002	0.05	0.01	0.15	0.003	0.10	0.003	0.04

Since the results are calculated from the average results of the extracts, there is no need for standart deviation ( $\pm$ SD) in this table.

Considering the percentages in dry plants in terms of THC, according to the data in Table 6, at least percentages of THC were obtained in Narlı strain. Even though it is still above the legal limits, if a poor production of THC rich in CBD is desired, the necessity of choosing the Narlı strain is proven in all circumstances.

### 3.2.3 Different strains which grown in same regions

See **Figure S2** to show which strains grow best in which region. Considering the polarity of the CBD and THC active ingredients, it was determined that the highest results were obtained with hexane and at the same time it was determined that the female species were more efficient than the male species, so we made the interpretation of which species should be cultivated in which region by considering these parameters. **Figure S2 A-B-C-D** shows the differences of different species in the same region.

Considering the Meram region, it is seen that the most productive strains in terms of CBD are Gökçeağaç and Narlı strains. At the same time, it is seen that THC is the lowest in Narlı strain (**Figure S2-A**).

It is seen that the most productive type in terms of CBD in Çumra region is Narlı, followed by Elnur strains with the highest percentage of CBD. In terms of THC, Gökçeağaç yielded the highest yield in Çumra region, while the lowest yield was obtained in Narlı strain (**Figure S2-B**).

It is seen that the most efficient strain in terms of CBD in the Beyşehir region is Narlı and the most efficient strain in terms of THC is Papatya (**Figure S2-C**).

It is seen in **Figure S2-D** that the most efficient strain in terms of CBD growing in Altınekin region is also Narlı strain, as in other regions, followed by Gökçeağaç strain. In terms of THC, it is seen that Elnur strain give very high result.

## 4. Conclusions

In accordance with the purpose of the study, the CBD and THC active ingredients extracted by the soxhlet extraction method from the leaves of four different cannabis strains harvested after planting seeds in four different regions in Konya region were analyzed by HPLC. Extract amounts and CBD / THC contents of dry plant both between regions and strains were examined. Based on the apolar character of the CBD and THC active ingredients, the extract yields obtained with the hexane solvent, which is the apolar solvent, are the highest, as expected. The lowest yields were obtained with ethanol solvent in all regions. In general, the highest extract yields were obtained from female species in accordance with the literature.

Although there are very low results in the literature in male strains, the yields obtained in our study are quite high. We can relate this to the close planting of male and female strains in the same environment. Because male and female species can pollinate by wind in the same environment and form an average strain. Under normal circumstances, male and female species would have to be planted in areas too far away to cause pollination. In the yields obtained by soxhlet extraction, the results of Gökçeağaç male strains using hexane solvent in Altinekin region (13.83%) and Gökçeağaç female strains in Beyşehir region (8.42%) were the highest results. The results of the Elnur female strains in Beyşehir region are in the second place with 11.27%. However, if evaluated in terms of the species with high results in all regions, the extract yields of the female of the Narlı strains obtained with hexane solvent are the highest (9.90%, 8.84%, 6.74% and 5.48%).

When all species are evaluated in terms of CBD content from HPLC analysis results after soxhlet extraction, all results in the Meram region are the highest. As it can be understood from here, if it is desired to obtain a CBD-enriched extract, it can be planted in Meram region. Narlı strain provided the highest yield (65.88%) in terms of CBD in accordance with the purpose of cultivation. When evaluated in terms of THC active ingredient, it was seen that each strain gave high yields in different regions after soxhlet extraction. Elnur strains gave the highest yields in Altinekin region (66.21%), Papatya strains in Beyşehir region (52.05%), Narlı strains (14.49%) and Gökçeağaç strains in Çumra (56.13%). It has been observed that the regions with the highest temperature and the lowest annual average humidity are more suitable for cannabis cultivation (Meram).

## **Declarations**

### **Author contributions**

The design of experiments, all the analysis, evaluations of results, and also drafting and writing manuscript were done by Umut Karğılı and Dr. Ezgi Aytaç.

### **Conflicts of Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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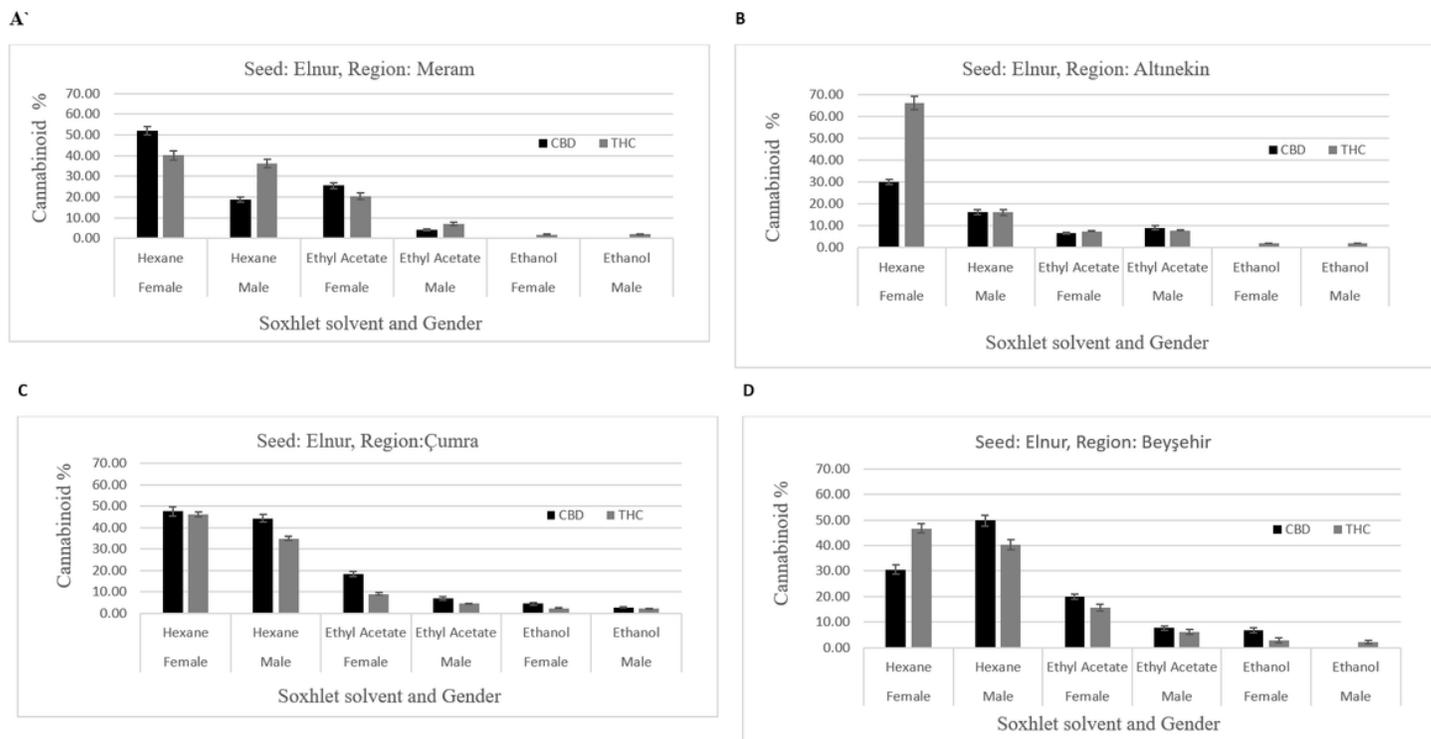
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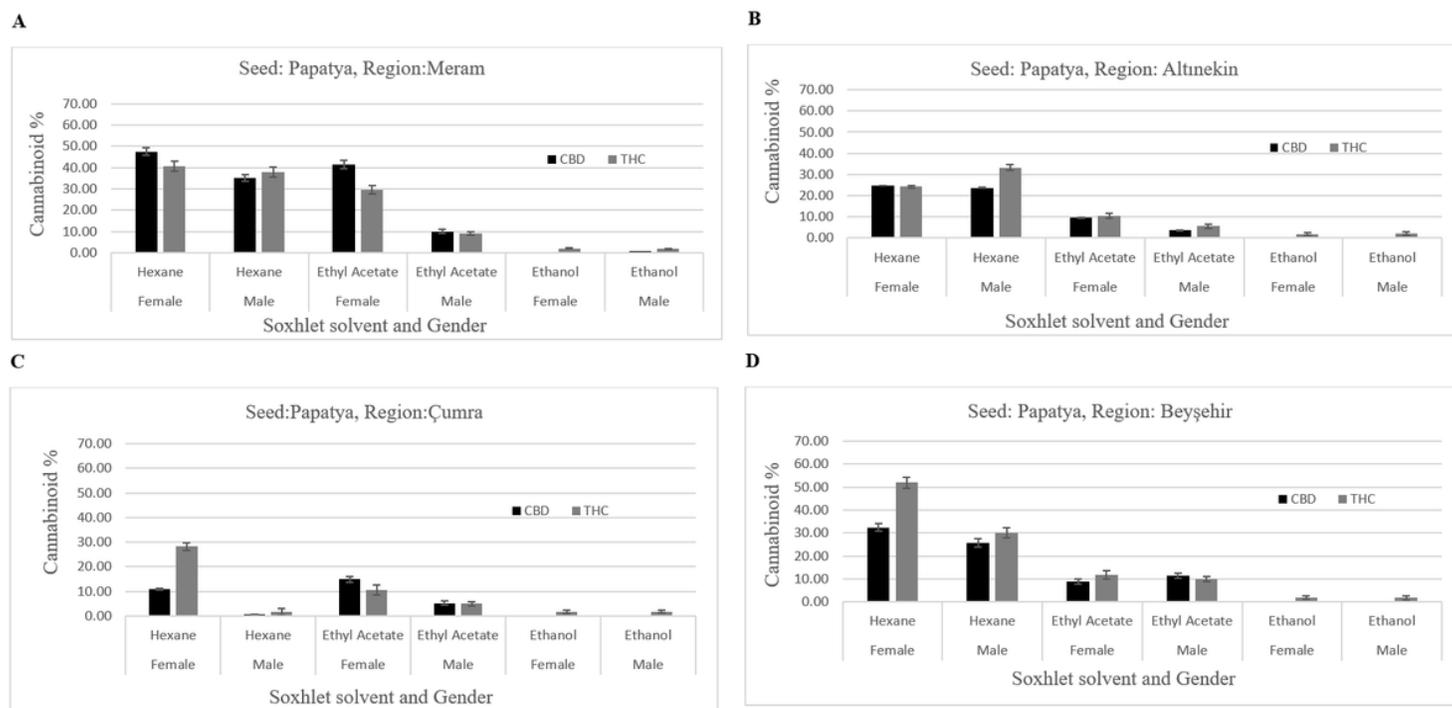
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# Figures



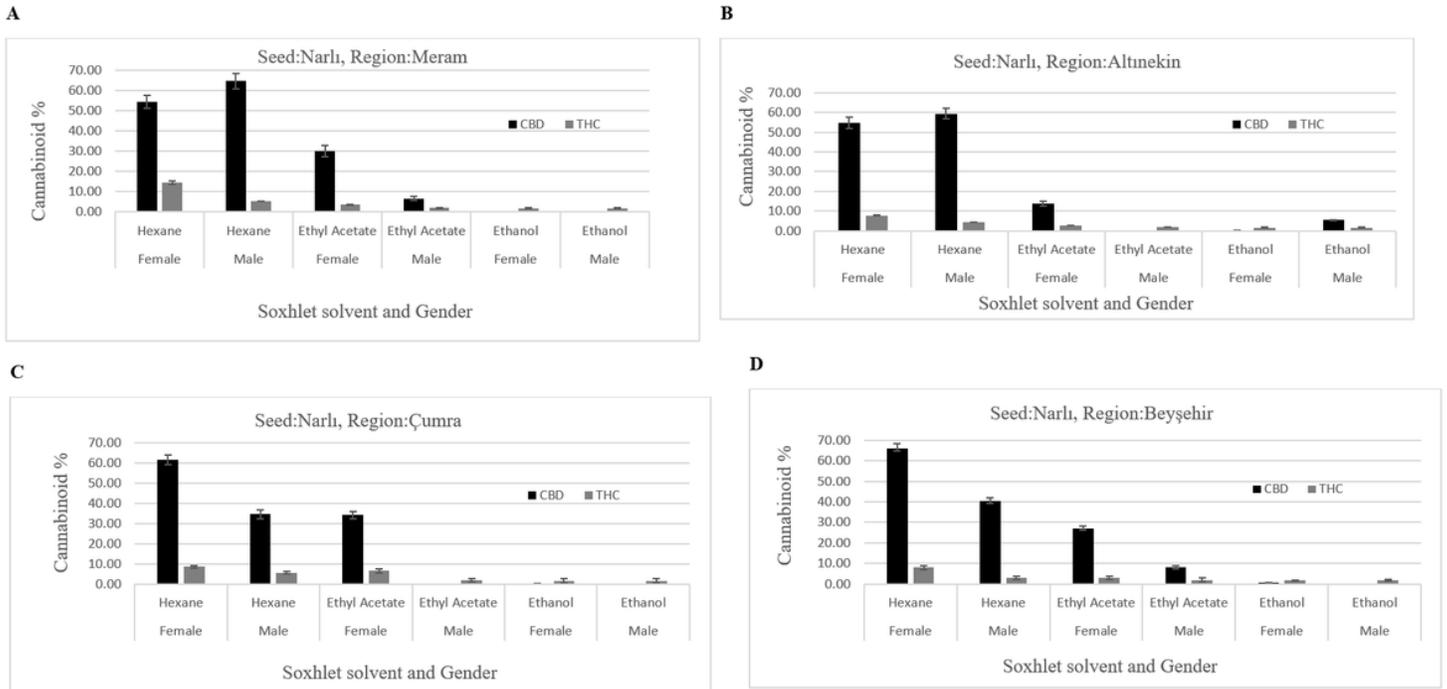
**Figure 1**

Cannabinoid contents (CBD and THC %) of Elnur strain which grown in different regions A:Meram B:Altınekin C:Çumra D: Beyşehir



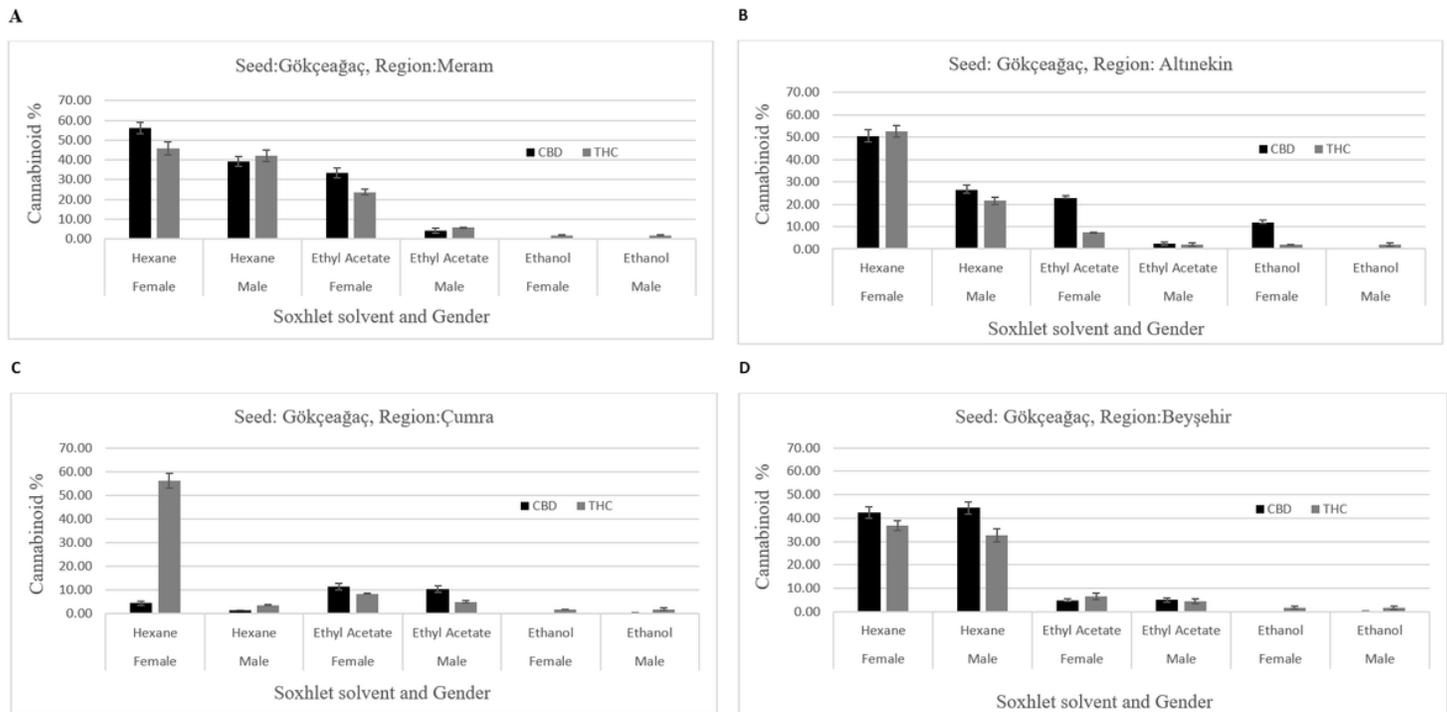
**Figure 2**

Cannabinoid contents (CBD and THC %) of Papatya strain which grown in different regions A:Meram B:Altınekin C:Çumra D: Beyşehir



**Figure 3**

Cannabinoid contents (CBD and THC %) of Narlı strain which grown in different regions A:Meram B:Altınekin C:Çumra D: Beyşehir



## Figure 4

Cannabinoid contents (CBD and THC %) of Gökçeağaç strain which grown in different regions A:Meram B:Altınekin C:Çumra D: Beyşehir

## Supplementary Files

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