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Review paper

Classification of Cannabis sativa L. variety Helena as industrial hemp: an analysis based on THC content

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Abstract

This study presents a forensic expert report aimed at determining that *Cannabis sativa* L. variety Helena does not belong to the types of *Cannabis sativa* that contain more than 0.2% THC, and therefore classifies as an industrial type plant rather than one used for narcotic purposes. The study was conducted at the request of the defense attorney of the accused, who is charged with the unauthorized production and distribution of narcotic drugs, in accordance with Article 215, paragraph 1 of the Criminal Code of the Republic of North Macedonia. The defendant legally imported the seed material into the country, along with a certificate confirming that it is industrial hemp, variety Helena. The Forensic Department of the Ministry of Interior, after performing analyses, extractions, and color tests on plant and seed samples, confirmed that the seeds do not contain psychoactive substances. However, it was concluded that if these seeds are planted, they can grow into Cannabis plants, and through processing, may produce substances used as narcotic drugs. The seized plant was destroyed 30 days after this evaluation, which made it impossible to conduct a quantitative determination of THC content after that period. Therefore, since the plant variety was already known, the only alternative was to confirm the THC content of *Cannabis sativa* L. variety Helena by referring to other scientific studies that have performed quantitative analyses of THC levels in this variety. The quantitative determination of THC is the key factor in classifying whether a cannabis plant falls under the category of narcotic (THC > 0.2%) or industrial hemp (THC ≤ 0.2%).

Key words: *Cannabis sativa* L., variety Helena, THC

Introduction

The *Cannabaceae* family is represented by three subspecies: *C. sativa*, *C. indica*, and *C. ruderalis*. Today, many researchers are convinced that the cannabis that grows commercially is C. sativa L, but the subvariety "sativa" should be known as hemp and the subvariety "Indica" should be called recreational cannabis or marijuana (Horne, 2020).

Industrial cannabis, also known as industrial hemp, refers to a group of *Cannabis sativa* L. varieties specifically cultivated for agricultural and industrial purposes. These varieties are primarily grown for their seeds and fibers and are clearly differentiated from recreational or medicinal cannabis based on their chemical composition (Owusu et al., 2021). These plants contain low levels of THC (generally less than 0.2–0.3%). In contrast, plants grown for narcotic or recreational use are characterized by high THC content, while those intended for medicinal use typically exhibit high levels of both THC and CBD (Breijyehet al., 2021). In most European countries, the maximum legally permitted THC content for cultivation is 0.2%, whereas in Canada the legal threshold is set at 0.3%.

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The main distinguishing factor related to the different intended uses of *Cannabis sativa* L. is the concentration of its two most well-known phytochemicals: tetrahydrocannabinol (THC) psychoactive compound, and cannabidiol (CBD), which is nonpsychoactive. Both compounds belong to the class of cannabinoids, a group of more than 100 secondary metabolites that are part of the terpenophenolic compound family, characteristic of all *C. sativa* L. plants.

Differences between Cannabis and Hemp

The cannabinoid biosynthetic pathway has been essential in demonstrating that the concentration of each cannabinoid in the plant is genetically determined. As a result, different genotypes with specific chemical phenotypes also referred to as "chemotypes" or "biotypes" exist, varying in the type and concentration of cannabinoids they produce (Broséus et al., 2010; Pacifico et al., 2006; Staginnus et al., 2014). Based on the content and ratio of THC and CBD, three principal chemotypes have been identified:

- Chemotype I is characterized by a low CBD/THC ratio (0.00–0.05, *%, w/w*), due to a high THC content (>0.3% dry weight of the female reproductive part at flowering). This chemical phenotype is also referred to as the "drug-type," "THC-predominant," or *C. sativa* L. subsp. *indica*, and the varieties in this group are typically cultivated for recreational or narcotic use.
- Chemotype II has a CBD/THC ratio close to unity (0.5–3.0, *%, w/w*), usually with a slight prevalence of CBD. Known as the "intermediate-type" or "THC-intermediate," this chemotype is mainly cultivated for medicinal applications.
- Chemotype III is distinguished by a high CBD/THC ratio (15–25, *%, w/w*), due to a high CBD content and a low THC level (not exceeding 0.2% dry weight of the female reproductive part at flowering). This chemical phenotype is also known as the "non-drug-type," "fiber-type," or *C. sativa* L. subsp. *sativa*, and the varieties in this group are primarily cultivated for industrial purposes—namely, fiber, seeds, and their derivatives.

Hemp seeds are particularly valued in the food industry for their unique composition, as they yield a protein rich isolate containing high levels of essential amino acids (Lan et al., 2019). Industrial hemp has traditionally been used in the textile industry. Compared to cotton, hemp fiber is a more economical material and offers several additional benefits. It is nonallergenic, highly absorbent, and provides natural protection against ultraviolet (UV) radiation (Fike, 2016). Hemp synthesizes approximately 500 biologically active compounds, which belong to several chemical classes such as cannabinoids (the most studied group), terpenes, hydrocarbons, nitrogenous compounds, carbohydrates, flavonoids, fatty acids, non-cannabinoid phenols etc. (Brenneisen, 2007).

The main cannabinoid found in industrial hemp is cannabidiol (CBD), a non-psychoactive compound known for its diverse pharmacological properties (Brighenti et al., 2017). CBD exhibits antioxidant and anti-inflammatory activity (Atalay et al., 2020), anxiolytic effects (Schier et al., 2012), antiepileptic properties (Devinsky et al., 2016), and notable antifungal and antibacterial activity (Berardo et al., 2024; Karas et al., 2020).

In addition to CBD, several studies have identified industrial hemp as a valuable source of polyphenolic compounds which contribute to its therapeutic potential. Another significant class of bioactive compounds present in hemp are terpenes (Nagy et al., 2019). Due to their lipophilic nature, terpenes demonstrate a wide range of biological activities, including anxiolytic, immunostimulatory, anti-inflammatory, analgesic, antioxidant, anticarcinogens (Gaggiotti et al., 2020), neuroprotective, and gastroprotective effects (Andre et al., 2016).

Furthermore, thanks to the development of specific analytical techniques (e.g., gas chromatography or gas chromatography–mass spectrometry), it is now possible to accurately and reliably measure the THC content in *C. sativa* L. plants, in order to distinguish between high-THC and low-THC hemp varieties.

The objective of this expert report was to determine whether *Cannabis sativa* L., variety Helena, belongs to the type of *C. sativa* cultivated for industrial purposes. This determination is based on its THC content being below 0.2%, in accordance with the threshold defined by European Union legislation.

Despite the fact that the defendant legally imported the seed material into our country, accompanied by a certificate confirming that it is *Cannabis sativa* L., variety Helena, the expert analysis conducted by the Ministry of Interior concluded that products derived from these seeds could potentially be used as a narcotic substance, marijuana, etc.

THC levels in *Cannabis Sativa* L., variety Helena

To achieve the objective of the study, the results of scientific research conducted by several researchers were reviewed. These studies included quantitative analyses aimed at determining the THC content in *Cannabis sativa* L. variety Helena.

According to Drinic et al. (2021), the results of the study showed that the commercial variety Helena can be designated as a cultivar with great potential for future use due to its chemical composition. Compared to other varieties, it is characterized by a higher content of nonpsychoactive CBD and a lower content of psychoactive THC (Table 1).

Table 1. Chemical composition of hemp (Cannabis Sativa L. variety Helena) total extract obtained by SC-CO2 extraction (Drinic et al., 2021)

			100 bar		
Nr.	Compound	RI	40 °C	50 °C	60 °C
15.	Cannabidiol (CBD)	2248.5	67.81	62.44	70.47
16.	Tetrahidrocannabinol (THC)	2326.3	1.44	1.40	1.88

According to the findings reported by Omar et al. (2013), the levels of cannabidiol (CBD) and tetrahydrocannabinol (THC) in supercritical CO_2 extracts of 13 different *Cannabis indica* samples showed considerable variation depending on extraction parameters such as pressure (100–250 bar), temperature (35–55 °C), and ethanol concentration used as a cosolvent (0–40%). The measured CBD concentrations ranged between 1 and 13 mg/g, while THC concentrations varied from 4.5 to 324 mg/g. In comparison, extracts from the industrial hemp variety *Helena* contain CBD concentrations roughly 100 times higher than the maximum levels found in the *C. indica* samples. This stark contrast emphasizes the superior CBD yield and overall quality of the *Helena* cultivar relative to *Cannabis indica*.

In the study titled *"Cannabinoid Content in Industrial Hemp (Cannabis sativa L.) Varieties Cultivated in Slovenia",* Tavčar et al. (2021) analyzed 15 industrial hemp varieties listed in the "Common Catalogue of Varieties of Agricultural Plant Species": Fedora 17, KC Dora, Monoica, USO 31, Helena, Santhica 27, Tisza, Tiborszallasi, Antal, Carmagnola, Finola, Kompolti hybrid TC, Ferimon, Novosadska, and Marina. The cannabinoid content (Δ8-THC, Δ9-THCA, Δ9-THC, CBC, CBDA, CBD, CBDVA, CBDV, CBGA, CBG, CBL, CBN, THCV) was determined using a validated HPLC method in bracts, other inflorescence parts, and whole inflorescences of each plant (n=3).

The highest total CBD+CBDA content in whole inflorescences was found in: Helena (2.4%), Tisza (1.9%), Antal (1.8%), Fedora 17 (1.6%), and Tiborszallasi (1.6%). The highest Δ9-THC+Δ9-THCA content was detected in KC Dora (0.9%), whereas in other varieties, it remained below 0.2%. CBG+CBGA was dominant in Santhica 27 (3.1%), with negligible levels of other cannabinoids. For varieties such as Fedora 17, USO 31, Tisza, Tiborszallasi, and Antal, the CBD content in whole inflorescences corresponded with the declared values. A consistent Δ9-THC/CBD ratio (*%, w/w*) was observed in Helena (1:31), Kompolti hybrid TC (1:33), Finola (1:29), Monoica (1:29), and USO 31 (1:33). A lower Δ9-THC/CBD ratio (*%, w/w*) was noted in Carmagnola (1:1 to 1:32), KC Dora (1:1 to 1:33), Tiborszallasi (1:1 to 1:30), and Tisza (1:1 to 1:31).

Based on theoretical data regarding the CBD/THC ratio, all analyzed *Cannabis sativa* varieties fall under the type of cannabis used for industrial purposes, as their CBD/THC ratio exceeds the threshold of 10–15 (*%, w/w*) (chemotype III)

According to Zeremski et al. (2018), in the study titled *"Cannabinoid Content and Fatty Acid Composition in Twelve European Varieties of Industrial Hemp",* industrial hemp (Cannabis sativa subsp. *sativa*) primarily contains CBD and little to no THC. The CBD content among the analyzed varieties ranged from 0% (Santhica) to 1.97% (Carmagnola) (Fig. 1).

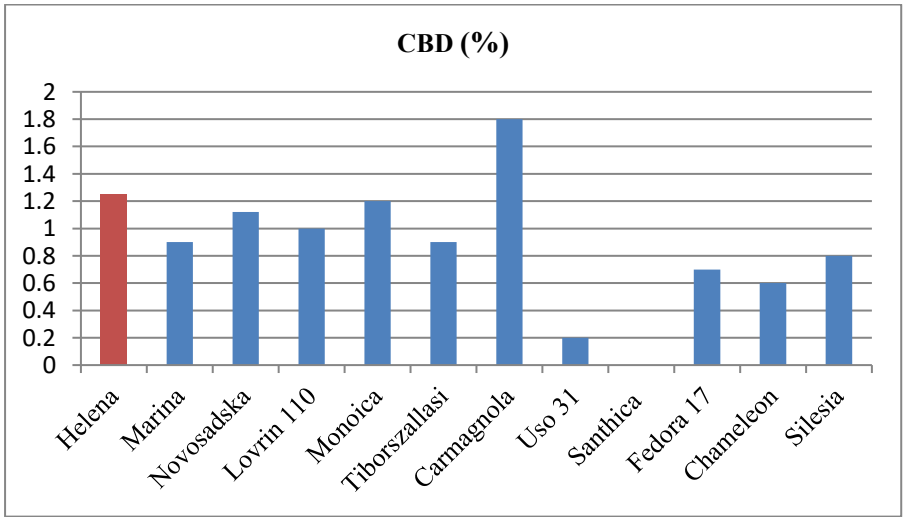

Fig. 1. Cannabidiol content (%) in fiber hemp flowering tops (Zeremski et al., 2018).

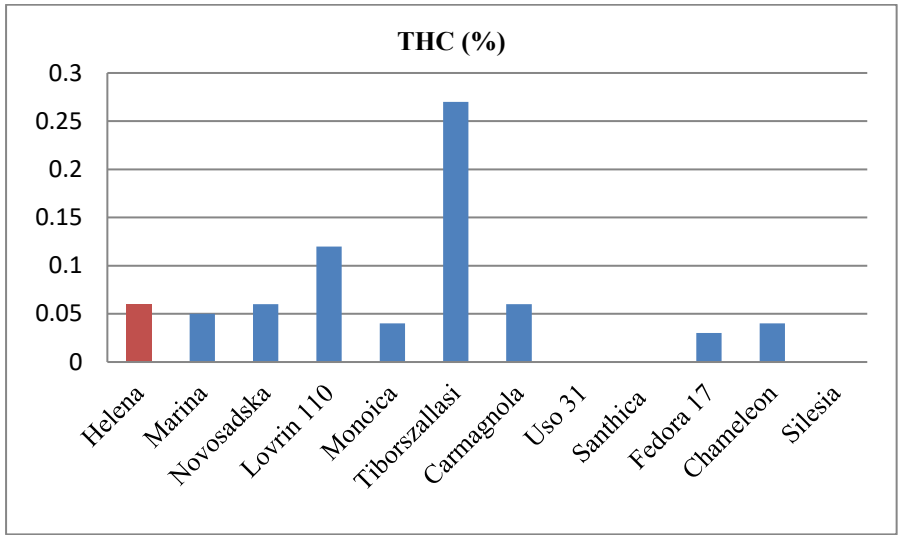

Figure 2. Tetrahydrocannabinol (THC) content (%) in fiber hemp flowering tops (Zeremski et al., 2018).

Table 2. Active compounds of hemp oil from the Helena variety (Institute of General and Physical Chemistry, Belgrade, 2018)

Compounds	%	Method
THC	0.16	GC-MS
CBD	42.0	GC-MS
CBC	1.8	HPLC
CBG	3.5	HPLC
Terpens		
Pinin	0.82	
Mirsin	0.27	HPLC
Linolin	0.69	

THC content ranged from 0% (USO 31, Santhica, and Silesia) to 0.29% (Tiborszallasi) (Fig. 2). In most EU countries, the current legal upper limit for THC content in hemp cultivation is 0.2% (Sarmento et al., 2015), while in Serbia, the threshold is 0.3%. The THC content in eleven out of the twelve analyzed hemp varieties was significantly below the EU legal limit (Fig. 2).

Below are presented the data from the quantitative analysis of *Cannabis sativa L.* cultivar Helena, conducted by the Institute of General and Physical Chemistry, Belgrade, Serbia. On 07.06.2018, a sample of hemp oil from the Helena cultivar was submitted for analysis to the aforementioned Institute. Using GC-MS and HPLC analysis, seven active components were identified: THC (0.16%), CBD (42.0%), CBC, CBG, and three terpene components (Table 2).

The expert report also included the results of the quantitative analysis of ten varieties of *Cannabis sativa L.*, including the variety Helena, conducted in 2015 by theInstitute of Field and Vegetable Crops, Novi Sad, Serbia (Table 3).

In the European Union, the reintroduction of hemp cultivation was formalized in 2013 through the implementation of EU Regulation No. 1307/2013, which permits the cultivation of Cannabis sativa L. strictly for industrial purposes, provided that the varieties used contain low levels of THC. According to this regulation, eligibility for marketing authorizations under the Common Agricultural Policy (CAP) is contingent upon the use of certified seeds from approved hemp cultivars specifically, Cannabis sativa L. varieties with a THC content not exceeding 0.2% of the dry weight of leaves and flowering tops (EMCDDA, 2018). This threshold is more restrictive than that of Canada, where the legal limit is 0.3%, and reflects the EU's emphasis on public health and safety. To support this framework, a variety of industrial hemp genotypes with THC levels below 0.2% have been selectively bred and registered. As of 2021, approximately 70 such cultivars are listed in the EU Plant VarietyDatabase as recognized agricultural species (EU databases, 2021; Pavlovic et al., 2017).

Table 3. Cannabinoids of ten varieties of *Cannabis sativa* L (Institute of Field and Vegetable Crops, Novi Sad (2015)

	Herb			Leaf			Flower			
Varieties	CBD (%)	THC (%)	CBG (%)	CBD (%)	THC (%)	CBG (%)	CBD (%)	THC (%)	CBN (%)	CBG (%)
	0.979	0.050		0.783	0.026		1.969	0.113		
	1.014	0.058		0.659	0.005		1.992	0.114		
Fedora 17	0.996	0.054		0.721	0.015		1.981	0.114		
	1.191	0.065		1.067	0.053		1.949	0.110		
	1.235	0.067		1.020	0.039		1.818	0.093		
Novosadska	1.213	0.066		1.043	0.046		1.884	0.101		
	1.499	0.080		0.866	0.037		1.610	0.074		
	1.325	0.072		0.934	0.043		2.160	0.125		
Secuieni	1.412	0.076		0.900	0.040		1.885	0.100		
	0.967	0.059		0.629	0.012		3.563	0.232		0.259
	1.042	0.063		0.541	0.000		3.292	0.212		0.242
Marina	1.005	0.061		0.585	0.006		3.427	0.222		0.250
	0.953	0.057		0.698	0.020		2.895	0.177		
	0.907	0.056		0.715	0.020		2.973	0.175		
Epsilon 68	0.930	0.057		0.707	0.020		2.934	0.176		
	1.229	0.070		0.855	0.015		2.021	0.114		
	1.303	0.071		0.866	0.017		1.804	0.094		
Helena	1.266	0.070		0.860	0.016		1.912	0.104		
	1.869	0.078		0.715	0.000		4.078	0.788		0.644
	2.044	0.089		0.706	0.000		4.160	0.722		0.626
Carmagnola	1.956	0.083		0.711	0.000		4.119	0.755		0.635
	1.238	0.072		0.532	0.273		3.394	0.651		0.000
	1.353	0.035		0.527	0.269		3.460	0.672		0.053
Futura 75	1.295	0.053		0.530	0.271		3.427	0.662		0.026
	1.153	0.021		0.933	0.008		2.176	0.122		
	1.085	0.050		0.907	0.010		2.141	0.111		
Felina 32	1.119	0.036		0.920	0.009		2.159	0.116		
	1.304	0.062		0.610	0.002		3.156	0.200		
	1.354	0.071		0.661	0.002		3.168	0.196		
Monoica	1.329	0.066		0.636	0.002		3.162	0.198		

National regulations across Europe reflect this EU-level framework, with certain regional distinctions. For instance, in Croatia, the Ministry of Agriculture adopted a regulation in 2013 permitting the cultivation of *Cannabis sativa L.* with THC levels below 0.2% solely for fiber production, seed production for animal feed, and seed reproduction. The 2019 amendment to the Anti-Drug Law recognized that CBD is not a drug. Back then, *Cannabis sativa* L. was removed from the country's list of narcotics. As a result, industrial hemp and its derivatives are regulated under the jurisdiction of the Ministry of Agriculture**,** rather than the Ministry of Health, and may be legally cultivated, processed, and traded as standard agricultural commodities (Law on the Suppression of Drug Abuse, Croatia, Official Gazette No. 80/13; 39/19)

In Serbia, hemp cultivation is regulated by the Law on Psychoactive Controlled Substances, adopted in 2010 and amended in 2018. The cultivation of industrial hemp is permitted using varieties listed in the Register of Recognized Varieties of Agricultural Plants, which includes cultivars such as *Novi Sad*, *Fedora 17*, *Helena*, *Marina*, and *Monoica*. Unlike most countries in the region that uphold a THC limit of 0.2%, Serbia allows up to 0.3% THC in industrial hemp. Permits for cultivation are issued by the Ministry of Agriculture in accordance with the 2013 regulation and cover activities such as fiber production, seed production for animal feed, further reproduction, processing, seed quality testing, and trade (Regulation on Hemp Cultivation Conditions ("Official Gazette of Serbia", No. 64/2013).

In this forensic report, the abovementioned results from the quantitative analysis of *Cannabis Sativa* L., variety Helena, showed that the Helena variety is characterized by a higher content of the nonpsychoactive compound cannabidiol (CBD) and a lower content of the psychoactive compound tetrahydrocannabinol (THC).Additionally, published results from scientific studies demonstrated that the THC content in *Cannabis Sativa L.*, variety Helena, was significantly lower than the legal limit established by the European Union (THC<0.2%).

However, the Court of Appeals, which was supposed to make a decision based on the submitted expert analysis, encountered two opposing positions:

- One position, which recognized that *C. Sativa* variety Helena is cultivated for industrial purposes.
- On the other hand, the results from the relevant institution within the Ministry of Internal Affairs (MIA), which stated that *C. Sativa* variety Helena is cultivated for industrial purposes.

As a result, the Court of Appeals decided to commission a supplementary expert analysis, which was conducted by a team of experts from the Department of Pharmacognosy at the Faculty of Pharmacy in Skopje.The expert reevaluation fully agreed with the professional stance that *Cannabis sativa*, variety Helena, represents industrial hemp, as the THC content is below 0.2%. Therefore, this variety cannot be cultivated for industrial purposes.

Conclusion

Cannabis sativa L. variety Helena is classified as industrial hemp, as its THC content is well below the legal limit set by the European Union (THC<0.2%). To determine whether a specific type of *Cannabis sativa* qualifies as industrial hemp or as a variety intended for narcotic use, a quantitative analysis of its THC content is essential.

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Резиме

Класификација на *Cannabis sativa* L. сорта Helena како индустриски коноп: анализа врз основа на содржина на ТХЦ

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Клучни зборови: Cannabis sativa L., сорта Helena, ТХЦ

Оваа студија претставува стручно мислење со цел утврдување дали *Cannabis sativa L*. сорта Helena ја надминува границата од 0,2% ТХЦ, која ја разликува индустриската сорта од онаа со наркотично дејство. Анализата е спроведена на барање на бранителот на обвинетото лице, за неовластено производство и дистрибуција на наркотични дроги, согласно Кривичниот законик на Република Северна Македонија. Имено, обвинетиот има легално увезол семенски материјал со сертификат за индустриската сорта Helena. Форензичката анализа, која вклучуваше екстракција и колориметриски тестови, потврди дека семето не содржи психоактивни супстанции. Сепак, беше констатирано дека ако семето се засади, растенијата може да произведуваат супстанции со наркотични својства. Растението беше уништено 30 дена по извршената анализа, што оневозможи спроведување на квантитативна анализа на содржината на ТХЦ. Со оглед на познатата сорта на растението, потврдата за ТХЦ-содржина се базираше на постоечки научни истражувања. Квантитативното определување на ТХЦ претставува клучен критериум за класификација на растението како наркотично (ТХЦ > 0,2%) или како индустриски коноп (ТХЦ < 0,2%).

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