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Original scientific paper

INFLUENCE OF AGROCLIMATIC CONDITIONS ON CONTENT OF MAIN CANNABINOIDS IN INDUSTRIAL HEMP (*Cannabis sativa* L.)

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In a six-year field experiment eight industrial hemp varieties were examined for Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) contents. The study analyzed the influence of growing degree days (GDD), soil temperature at 5 cm, air humidity, and growing season precipitation on the levels of the main cannabinoids in this crop.Agroclimatic conditions do not influence THC and CBD contents in industrial hemp in the same way. THC synthesis and accumulation are under the significant positive influence of GDD and air humidity and under the negative influence of precipitation, while soil temperature at 5 cm has no significant effect on it. Soil temperature at 5 cm has a significant positive effect on the CBD content, as do GDD. Precipitation has a negative influence on the CBD content of industrial hemp, while air humidity has no influence on it.

Key words: agroclimatic conditions, cannabinoids, CBD, industrial hemp, THC

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INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) belongs to so called alternative crops grown on limited are by using specific crop production technology by which considerably higher profit is obtained from the unit of area as compared to so called conventional crops (i.e. corn, wheat etc.) (BERENJI *et al.* 2011; DAHLBERG *et al.* 2011; BERENJI and SIKORA, 2001). The term „industrial hemp“denotes hemp plants that are grown for fiber or grain. Hemp seed oil is a higly valued edible oil (BERENJI *el al.* 2001; DIMIĆ *et al.* 2006). The psychoactive components (cannabinoids) present in the upper parts of the hemp plant are used in medicine (HORVAT *at al.* 2005) but at the same time are considered to be an important reason for the decline of industrial hemp cultivation worldwide in the course of the 20th century (BERENJI *et al.* 2002).

Cannabinoids are one of the 400 different chemical substances that have been isolated from hemp. These terpenophenolic substances are accumulated mainly in the glandular trichomes of the plant. The most abundant of over 60 different cannabinoids are cannabidiol (CBD) and Δ^9-tetrahydrocannabinol (THC) (HOLLEY *et al.* 1975). THC is the only psychoactive component in hemp, and it has no adverse effects on fibre or grain quality (MECHTLER *et al*. 2004). The THC content in the upper part of hemp plants is limited to 0.2% by EU regulations (Council Regulation /EC) No. 1420/98).

The chemical phenotype, or chemotype, of hemp is determined by the main cannabinoid content in the inflorescence dry matter (HILLING, 2002). SMALL and BECKSTEAD (1973) described three chemical phenotypes on the basis of specific cannabinoid ratios and quantities: Chemotype I (the drug type), with a THC amount of over 0.30% and a CBD content of less than 0.50%; Chemotype II (the intermediate type), in which CBD is the prevalent cannabinoid but in which THC is also present at various concentrations; and Chemotype III (fiber hemp), with THC of less than 0.30% and CBD of over 0.50%. Contemporary industrial hemp cultivars generally have psychoactive potency that is below the acceptable limit (BERENJI and SIKORA, 1996; FINTA-KORPEL'OVÁ and BERENJI, 2007).

Cultivated plants are under the strong influence of the environment and production technology during their life cycle (LATKOVIĆ *et al.* 2011). The saame applies to the total amount of cannabinoids synthetized by the fibre hemp plant (MANDOLINO *et al*: 2003), but its variability across environments is still not fully defined.

The aim of the present study was to evaluate the effects of growing degree days (GDD), soil temperature at 5 cm, air humidity, and growing season precipitation on THC and CBD contents in industrial hemp.

MATERIALS AND METHODS

A field experiment was carried out on chernozem at Bački Petrovac (45°20´N 19°35´E) over a period of six years (1999, 2000, 2001, 2003, 2004 and 2005). The soil at the site has the following properties: organic matter content – 2.70-2,90%, pH in H_2O – 8.56-8.68, P_2O_5 – 45.2-47.0 mg/100g, and K_2O – 54.0-56.8 mg/100g.

Before sowing 60 kg ha^{-1} of nitrogen fertilizer were applied. Along with the existing 0.194-0.209% of total nitrogen in the soil, this amount of fertilizer represents the optimal level of macroelements for growing industrial hemp in the region where the experiment was located (AMADUCCI *et al.* 2002).

The experiment was designed based on recommendations for growing industrial hemp provided by STRUIK *et al.* (2000). Every year the sowing was done in the first ten-day period of April. Using 60 kg ha^{-1} of seed and a 12.5 cm distance between rows, a density of 240 plants m^{-2} was reached.

Approximatelly 20 plants were sampled successively at the end of flowering female respective monoecious plants (MEDIAVILLA *et al.* 2001). Each plant was cut at its upper third and air-dried at ambient temperature to a residual humidity of less than 12%. Material for laboratory analysis included leaves, blossoms, small structural parts of the inflorescence, and bracts, which were separated manually from stems and seeds. All the samples were individually analysed by gas cromatography to quantify THC and CBD using the procedure of GRASSI and RANALLI (1999).

The field experiments included five conventional monoecious (Futura 77, Ferimon 12, Fedora 19, Beniko and Bialobrzeskia) and two dioecious (Novosadska and Lovrin 110) European industrial hemp varieties as well as one dioecious variety of Hungarian provenance (Tiborszallasi).

Temperature, air humidity, and precipitation were measured at day level at the local meteorological station located at a distance of approximately 500 meters away from the experiments. The total GDD was calculated as the sum of average temperatures of all days in the entire growing period. For the presentation of the results correlations and regression analysis were used.

RESULTS AND DISCUSSION

Relative to the long-term average air and soil temperatures, our trial included one average (1999), two cooler (2000 and 2003), and three warmer growing seasons (2001, 2004, and 2005). Over the studied period, average daily humidity during the period between emergence and technical maturity ranged from 46.6% in 2000 to 62.9% in 1999. Relative to the long-term average value for the locality studied (52.7%), the years 2004 and 2005 could be regardes as average, 1999 and 2001 were extremely wet, while 2000 and 2003 can be considered dry. Looking at the long-term average for growing season precipitation (401.8 mm) at the site of the study, the years 2000 and 2003 were extremely dry, 2001, 2004 and 2005 were wet, while 1999, with 389.0 mm of growing season precipitation, was an average year (Table 1). The wide variation of agrometeorological parameters during the trial has made it possible to obtain a detailed picture of the effects these factors have on the levels of the main cannabinoids in industrial hemp.

Based on the six-year mean value and the relation between the THC and CBD contents, the varieties Futura 77, Lovrin 110, and Tiborszallasi can be classified as intermediate according to the classification by SMALL and BECKSTEAD (1973), while the other five varieties can be categorized as the fiber type.

Table 1. Average GDD (°C), soil temperature at 5 cm (°C), air humidity (%), and sum of precipitation (mm) from emergence to technical maturity of industrial hemp at the Bački Petrovac site.

	Year						Average	25 year
	1999	2000	2001	2003	2004	2005		average
GDD (°C)	1932,8	2116,8	1855,9	2116,4	1857,4	1852,3	1955,3	1954,6
ST (°C)	21,5	23,5	20,3	22,8	20,4	20,4	21,5	21,9
Air humidity (%)	62,9	46,6	57,2	46,8	54,1	53,5	53,5	52,7
Precipitation (mm)	389,0	103,0	569,6	230,2	487,8	551,9	388,6	401,8

ST soil temperature at 5 cm (°C)

The value of the standard error and standard deviation in Table 2 are in agreement with the findings of MANDOLINO *et al.* (2003) indicating that the total amount of cannabinoids synthetized by industrial hemp is under strong environmental influences.

Table 2. Mean and range values of the levels of the major cannabinoids in industrial hemp at the Bački Petrovac site.

	THC (%)				CBD (%)			
	Max	Min	Mean ± SE	SD	Max	Min	Mean ± SE	SD
Novosadska	0,301	0,061	0,137±0,008	0,081	1,763	0,310	1,125±0,050	0,467
Futura 77	1,000	0,045	0,494±0,032	0,341	3,261	1,011	2,039±0,071	0,738
Ferimon 12	0,250	0,060	0,133±0,008	0,077	2,320	0,430	1,444±0,064	0,630
Fedora 19	0,670	0,022	0,264±0,024	0,226	2,228	0,568	1,524±0,052	0,513
Lovrin 110	1,000	0,040	0,581±0,034	0,336	2,903	0,560	1,406±0,076	0,751
Beniko	0,541	0,035	0,252±0,020	0,183	2,509	0,715	1,592±0,059	0,604
Bialobrzeskia	0,264	0,033	0,120±0,008	0,076	1,920	0,760	1,304±0,040	0,413
Tiborszallasi	0,800	0,137	0,550±0,019	0,211	2,450	0,689	1,474±0,060	0,574

Correlation analysis presented in Table 3 indicates that agroclimatic conditions did not have the same type of influence on THC and CBD contents in industrial hemp. THC synthesis and accumulation were under the significant positive influence of GDD and air humidity and under the negative influence of precipitation, while soil temperature at 5 cm had no significant effect on the two processes (Figure 1). In the case of CBD, soil temperature at 5 cm had a significant positive effect on the CBD content, as did GDD. Precipitation had a negative influence on the CBD content, while air humidity had no influence on this trait (Figure 2).

BÓCSA and KARUS (1998) report that industrial hemp plants require a total heat quantity over the growing period of 1,900-2,000 GDD from germination to technical maturity. In our experiment we noticed an increase in the cannabinoid contents (both THC and CBD) with increasing temperature. The increase was more significant in the case of CBD than in that of THC.

Table 3. Correlation coefficients (r) GDD (°C) soil temperature at 5 cm (°C), air humidity (%) and sum of precipitation (mm) from emergence to technical maturity of industrial hemp at the Bački Petrovac site.

	THC (%)	CBD (%)
GDD (°C)	0,15*	0,29*
Soil temperature at 5 cm(°C)	-0,05	0,72**
Air humidity (%)	0,33*	0,02
Precipitation (mm)	-0,56**	-0,74**

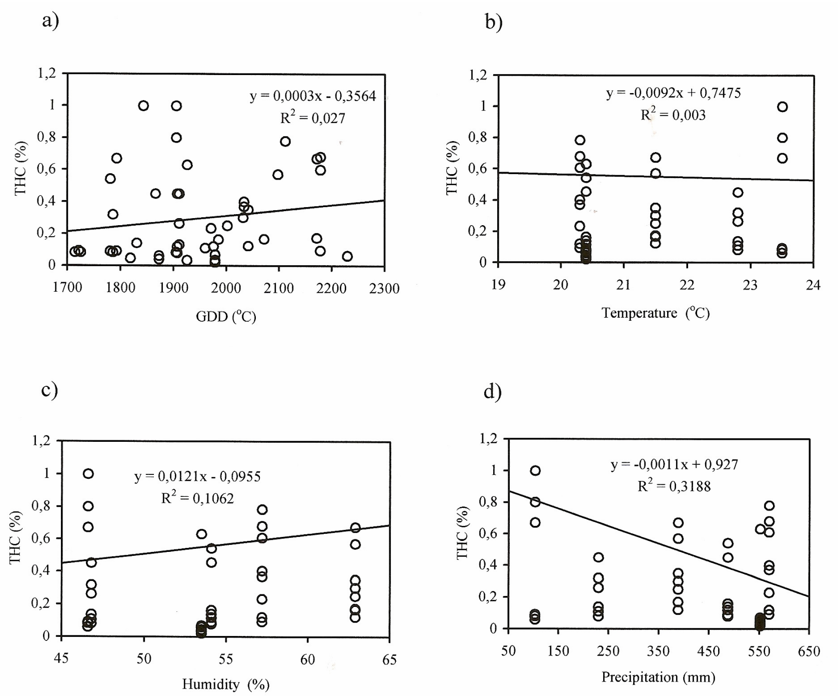

Figure 1. Regression of GDD (a), soil temperature at 5 cm (b), air humidity (c), and sum of precipitation (d) from emergence to technical maturity on THC content in industrial hemp at the Bački Petrovac site.

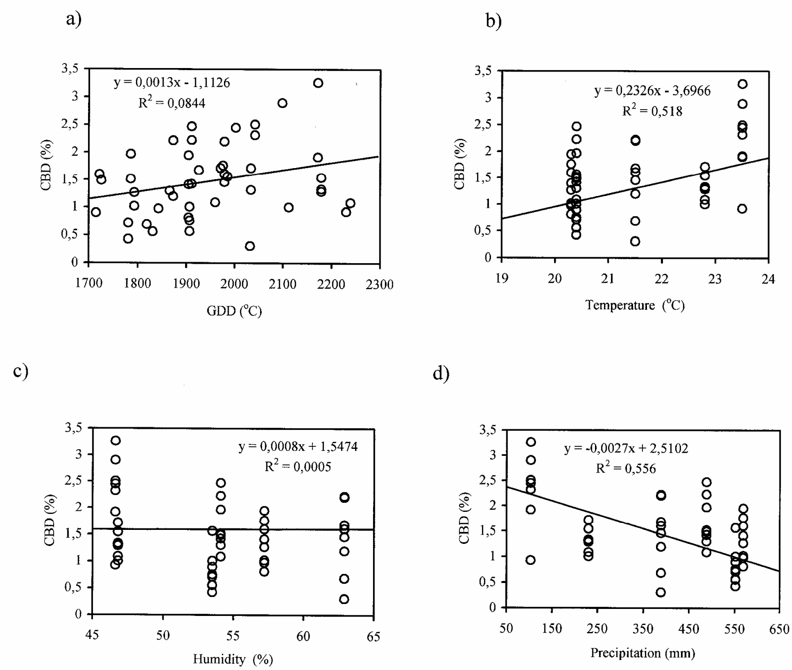

Figure 2. Regression of GDD (a), soil temperature at 5 cm (b), air humidity (c), and sum of precipitation (d) from emergence to technical maturity on CBD content in industrial hemp at the Bački Petrovac site

The direct precursor for THC and CBD synthesis is cannabigerol (CBG) (de MEIJER *et al.* 2003). The results of our research confirm that soil temperature has a significant effect on CBD synthesis and accumulation and no influence on THC content. The assumption is that increasing soil temperature stimulates metabolic processes leading to the synthesis of CBD or suppresses the synthesis of THC.

According to PATE (1999), temperature may play an important role in determining the cannabinoid content, but perhaps only through its association with moisture availability. When growing season precipitation increases to over 350 mm, the cannabinoid content significantly decreases. These results are in agreement with those reported by BÓCSA and KARUS (1998) according to which industrial hemp requires 250-300 mm of precipitation or an adequate quantity of water during the vegetative period. Precipitation affected the CBD content (R^2=0.5560) more significantly than it did the THC content (R^2=0.3188).

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UTICAJ AGROKLIMATSKIH USLOVA NA SADRŽAJ GLAVNIH KANABINOIDA U INDUSTRIJSKOJ KONOPLJI (*Cannabis sativa* L.)

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I z v o d

U poljskim ogledima je tokom šest godina osam sorti industrijske konoplje ispitivano na sadržaj Δ^9-tetrahidrokanabinola (THC) i kanabidiola (CBD). Analiziran je uticaj sume temperatura (growing degree days GDD), temperature zemljišta na 5 cm, vlažnosti vazduha i sume padavina tokom vegetacionog perioda na sadržaj glavnih kanabinoida.

Agroklimatski uslovi ne utiču u istoj meri na sadržaj THC i CBD u industrijskoj konoplji. Sinteza i akumulacija THC protiče pod značajnim pozitivnim uticajem GDD i vlažnosti vazduha i negativnim uticajem sume padavina. Temperatura zemljišta na 5 cm nema značajnog efekta na sadržaj THC, ali zato signifikantno utiče na sadržaj CBD, isto kao i GDD. Suma padavina ima izražen negativan efekat, dok je uticaj vlažnosti vazduha na sadržaj CBD u industrijskoj konoplji zanemarljiv.

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