Agronomic impacts on the performance of active ingredients of hemp (*Cannabis sativa* L) plant

Laura CZERŐDINÉ KEMPF[1] – Zuzana FINTA[2] - Ákos TARNAWA [1]

1: Institute of Crop Production, 2100 Gödöllő, Páter Károly u. 1.; E-mail: Kempf.Lauraaa@gmail.com

2: Agromag Ltd, 3356 Kompolt, Fleischmann u. 4.;

Abstract: The presentation comprises a literature review in relation with the application impacts on the cannabidiol content of fibre hemp plants. Since cannabidiol is a highly valuable natural compound used for various purposes from phamaceutical fields to direct chemical applications, there is a need to get acquainted with the agronomic conditions of its crop physiology.

Keywords: Cannabis sativa, cannabidiol (CBD), hemp active ingredients, agronomic impacts, non-psychoactive cannabinoids,

Hemp is the most important of our fibre crops under temperate climate, but the purpose of its cultivation extended in the last few years thanks to its highly valuable natural compounds.

The origin of hemp is in Central Asia. From there the plant came to north and central Europe in the 7th century B. C. but through several ways. It is said that hemp was brought by the Scythians to the regions north of the Black Sea and the mouth of the Danube by the direction via Russia. Hemp has been known since about 450 B. C. in the Mediterranean countries, where it is also spread by way of Asia Minor. (Bredemann et al. 1956)

The oldest information about the plant, its production of bast-fibers from the stem has been known to the Chinese from about 2800 B. C. The seeds were used for human consumption just later. The medical purposes have been discovered in India, where the plant was cultivated for since 900-800 B. C. as a source of a drug. In North-Africa since the mediaeval has been cultivated exclusively for hashish. (Bredemann et al. 1956)

The main varieties performed under varied climatic conditions and developed as a result of natural selection. It was mostly influenced by very different lengths of day and vegetation period. Although hemp is a very old cultivated crop in great part of Europe, it could be described as a wild plant cultivated by man, rather than as a genuine, cultivated crop. (Bredemann et al. 1956)

Thanks to that the varieties were grown for a long time in lack of systematic breeding work it has a number of unfavourable characteristics of wild plants until the breeding started in Hungary in the 1950's. (Bredemann et al. 1956) One chemical constituents of Cannabis was undesirable used as a fibre crop.

Up to 2005 489 natural constituents of *Cannabis sativa* L. have been identified (Rishi 2011), of which 70 are cannabinoids, the others are terpenes, hydrocarbons, sugars, proteins, enzymes, flavonoids, vitamins, pigments, elements (Gambaro 2002). Among the 70 known cannabis cannabinoids, the most important in terms of pharmacological relevance are tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN).

THC, the primary psychoactive component of cannabis exists in two isomeric forms known as Δ9-THC and Δ8-THC depending upon the position of the double bond in the

C-ring (Holler et al. 2008), and its structure was elucidated by Mechoulam, et al. in 1964. According to Mehmedic (2010) at least 100 different cannabinoids have been described.

Cannabidiol was isolated by Adams et al. (1940), from freshly harvested North American hemp. It is a dihydrophenol, which is not the active principle of cannabis either (Beam 1911).

According to Grlic (1962) CBD constitutes approximately 40% of the cannabinoids in cannabis organic extracts. Cannabinoids with lower importance are cannabigerol (CBG), cannabichromene (CBC), cannabicyclol (CBL), cannabielsoin (CBE), cannabinodiol (CBND), cannabitriol (CBT) and other miscellaneous compounds (Rishi 2011).

The most important parts of the plant in terms of the production of the active ingredients are the leaves and bract covered by glandular hair. It is the location where the Δ^9-tetrahydrocannabinol with other cannabinoids and terpenes are synthetizing (Hammond & Mahlberg, 1977).

Nowadays there is a need on the field of medicine, cosmetics and healing for some of the cannabinoids produced by Cannabis plant. In many countries of the World - also in Hungary - nether the recreational use, nor the medical marijuana usage is not allowed due to the psychoactive effect of the THC. Therefore the Hungarian hemp breeders strive to increase the amount of CBD among the valuable, non-psychoactive cannabinoid in the new Hungarian industrial hemp varieties. In parallel the amount of THC may not rise, moreover continuously stay under 0,2% (Implementing Regulation of the Commission 809/2014/EU). With the efficiency of the THC-reductional breeding in the 1960's carried out in Hungary, the other cannabinoids like CBD, CBDA, CBN, CBG also decreased and now should be increased again as required. Bredemann et al. were reporting in 1956, that hashish is the resin produced by the glandular hairs of the female inflorescence, and this resin contains at least three chemically-related substances: cannabinol (CBN), tetra-hydrocannabinol (THC), and cannabidiol (CBD). "Of these, cannabidiol found in the greatest quantity, has no narcotic effect; cannabinol is weak or inactive; but tetrahydrocannabinol, a derivative of cannabidiol, is the principal narcotic agent (Todd 1940, 1942, Bergel et al. 1938, Adams et al 1940a, Adams et al. 1940b, Bose & Muckerji 1943, Wollner et al. 1942)."

Bredemann et al. in 1956 were also reporting about problems of modern hemp breeding, and the need of hemp varieties containing little or no hashish: the elimination or reduction of the hashish content, which has remained the same in the cultivated as in the wild varieties. Along with many others they name the problem of high cannabinoid content causing an essential breeding goal because even the fibre hemps can produce so much hashish on their female inflorescence that they can be misused for producing narcotics.

They suggested that it must be possible to eliminate these undesirable narcotics by breeding. The concept was to look first for mutants which lack only one hashish component and the three different substances that hashish contains has to be followed in breeding. Judging by experience with other cultivated plants, mutants which lack only one of the hashish components can be found more frequently than mutants or combinations which lack all three. After breeding varieties of the Indian hemp plant which have very little or no hashish, the next step towards a non-psychoactive variety is to produce strains which

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lack all three by cross-breeding these varieties (Bredemann, G. 1956). *Cannabis indica* is rather used with purpose of the active ingredients than the other species caused by their higher cannabinoid content, but on the hemp seed market is also available some variety containing C. sativa and C. ruderalis strains. Defining taxonomic categories of the genus cannabis is still controversial.

According to Soó (1953), Simon (1992) and Borhidi (1998) the *Cannabis* fall under/ belongs to the phylum of Angiospermae, the class of Dicotyledones, the order of Rosales, and the family of Cannabinaceae. Besides the *Cannabis* L. genus only the hops (*Humulus* L.) genus belongs to this family.Previously hemp and hops were both classified into the *Moraceae* or *Urticaceae* family (Jávorka 1925).

Today it is generally accepted that hemp and hops together forms a separate family, the *Cannabaceae* = *Cannabinaceae*. The two related genera, hemp (*Cannabis* L.) and hops (*Humulus* L.) belongs to this family. According to this conception the genus/tribes has not races; only one species named by Linnaeus *Cannabis sativa*, which is divided into multiple varietas (Small 1975, Small & Cronquist 1976, 1999 Ranalli, Iványiné 2005):

- *Cannabis sativa* var. *vulgaris* (common, cultivated hemp)
- *Cannabis sativa* var. *indica* Lam. (Indian hemp)
- *Cannabis sativa* var. *indica* Lam. subvar. *gigantea* (giant hemp)
- *Cannabis sativa* var. *Ruderalis Janisch* (the so-called weed hemp).

According to the opinion of Szizov and Szerebrjakov, there are two species: *Cannabis indica* and *Cannabis sativa*. Within the *Cannabis sativa* species there are two subspecies, *Cannabis sativa* L. Szerebr. subsp. *spontanea* and *Cannabis sativa* L. Szerebr. subsp. *culta*, which are accepted in cultivation.(Mándy & Bócsa 1962)

The majority of the handbooks in Hungary also describe the spontaneous growing hemp as a separate subspecies (*C. sativa* ssp. *spontanea*), and definitely distinguish from the hemp shape that got mad and grow naturalized, which is also classified as subspecies taxa (*C. sativa* ssp. *sativa*) cit in Benécsné (2003). Due to the geographical environment emerged different geographical races: Northern hemp, Central Russian Hemp, Southern (Mediterranean Hemp and Asiatic Hemp race (Láng, 1976). There are significant morphological and physiological differences between geographical races, but despite this, it cannot be broken down into further taxonomic categories, because there is no genetic barriers between the most diverse forms; the dwarf-growing northern hemp and the non-ripening East Asian hemp both have 2n = 20 chromosomes. (Bócsa 2004).

The diversity at the hemp taxonomy can be caused by the genetic and agronomic background as well. Very important questions remain regarding field-scale systems to produce cannabinoids. An optimal agronomical proposal for cannabinoid production at *Cannabis sativa* has not been defined. These should include variety collection, nutrition, soil preparation, sowing, plant care, harvest and crop management of the industrial plant in terms of the newly emerged demand for the cannabinoids. The variety collection in Hungary includes varieties and hybrids exclusively from the *Cannabis sativa* species. All of the Hungarian varieties and hybrids, used for fiber, energy plant, or crop are proper for cannabinoid production as well. The amount of the two most important cannabinoids, the Δ9-tetrahydrocannabinol level is between 0,1% and 0,2%, but definitely under 0,2%, the cannabidiol is about 1-3% depending on the variety.(http1) The maximum amount of Δ9-tetrahydrocannabinol is regulated by law, and growing any species with higher content of the psychoactive ingredient is forbidden.

Coffman and Gentner (1975) declare that Turkish fibre variant of *Cannabis sativa* did not change cannabinoid profiles when the variants were grown in different environments; but also say, that nitrogen content in vegetative parts of the plant has been thought to correlate positively with its THC content. An experiment was performed in Hungary using Kompolti Hibrid TC variety to get knowledge about the effect of nitrogen fertilization on THC content in hemp leaves. The fertilizer treatments were 150, 450 and 600 ppm N, from NH_4NO_3. These experiments show that the THC content of leaves decreases with increasing N doses. The decrease was significant in the case of the highest N dose. There was a significant increase in fresh weight of shoot (80-130%) and plant height (28-39%) due to N supplementation.

This phenomenon is favourable for agricultural production, because nitrogen fertilization will increase stem yield and simultaneously decrease THC content of the plant significantly. (Bócsa, I., P. Máthé, and L. Hangyel 1997) According to the results of further research, the amount of THC and CBD are increasing simultaneously during the ripening (Kempf 2015), so the predictable decrease of both of cannabinoids occurred by high dose N fertilization is not preferred in terms of CBD-usage. As the N fertilization is in a negative correlation with the THC content; it seems to be possible, that higher N amount is also in a negative correlation with CBD. It could be a further aim of our field-experiments. By high density the crops generates rare, unbranched population, at lower density the hemp evolves thick, branched, coarse stems.Agromag Ltd, the biggest hemp propagating company in Hungary offers a 12, 24 or 70 cm row spacing for the Hungarian species depending on the breeding purpose. (Agromag 2015)According to Agócs et al. (1962) the seed yield in rare position will be big thanks to the richly branching: the optimal field size for a seed purpose plant is 0,35-0,49 m^2 in average of different years. It is practically 50-70 cm plant distance by 70 cm row spacing. However, sowing rates and plant size has a connection, it is unknown if increased production of female flowers as would be expected with decreased plant densities would result in increased yields of CBD, thanks to stress from competition.

- Bill Drake (1986) proposes by transplanting in rows the Southwest-Northeast or Southeast-Northwest row orientation, rather than the North-South or East-West. Because of blocking some of the sun, in the north, where the sun's rays fall slanting upon the earth, it can mean a significant reduction in vigour and therefore in crop yield at harvest.
- The same handbook mention pruning for mass protection, as the most common form to force side growth. Cutting off the head between the fifth and sixth sets of branches cause multiple shoots to develop, one on each side on each node. Due to its outstanding ability to suppress weeds during its vegetation period, the thick-sown hemp requires no herbicides during that time. Weeds are eradicated during soil preparation for sowing. In row separation the plants can not completely overshadow the soil, and may require the use of herbicides or hoeing. (Bócsa I., Karus, M. 1997, Bócsa I. 1996)
- The technical length of the bust fibre is a value measure property in the fiber industry, the branched stem is even not advantageous by dual usage: utilize the fiber and the

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seed or fibre and flowering terminal branches. (Agócs P. 1962; Finta Z. 2012)

- According to Geoffrey (2004), the contents of the key constituents of Cannabis sativa L., cannabinoids, terpenoids and flavanoids varies depending on the plant genetics, time of harvest and drying conditions.
- The time of harvesting has a high importance on CBD content according to our latest research connected with the varieties. (Kempf 2015) The early ripening dioecious candidate variety used for seed yield did not show a statistically proven difference in cannabinoid content between the flower and the bract around the seed after ripening. The result of the other, late ripening monoecious variety attended in the experiment differs: the CBD content measured in the bract was higher, than the CBD content in the flower samples. It shows that the amount of this non psychoactive cannabinoid is rising during the ripening in the late ripening monoecious variety. These characteristics are in connection with the harvest time: by this variety the harvesting – also for cannabinoid usage - is recommend after the full ripening of the seed.

The time of harvesting has also significance such as the harvested part of the plant. An experiment by Bócsa et al. (1997) shows, that the amount of THC was significantly different in leaves from various plant regions: leaves of the plant axis, the side branches and the plant top. THC was highest in leaves near the shoot tip and on side branches, and lowest in oldest leaves. (Bócsa, I., P. Máthé, and L. Hangyel 1997). The cannabinoids are in acidic form in the plant part. The decarboxylation, as a postharvest treatment is essential for transforming the acidic form into a neutral form. Carboxylation can be carried out by heating or burning the leaves. The duration and temperature of treatment may indicate different efficiency: the higher, 137 oC / 1 hour treatment is more efficient than the 45 ^{o}C / 24 hour treatment (Kempf 2015).

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