

Changes in the content of terpenes, cannabinoids and essential oil in the organs of industrial hemp *Cannabis sativa* L. subsp. *sativa* during growth in field conditions

Currently, a plant that is experiencing its renaissance in Europe is industrial hemp. Due to the similarities between industrial hemp and the medical type of hemp, industrial hemp production was severely restricted years ago in most countries, destroying centuries of science. In Poland, starting from the 1960s, the area of cultivation gradually decreased, and the complete collapse of the industrial hemp market took place in free Poland in the 1990s. The national downward trend was caused by the collapse of the spinning market and the mass liquidation of the retting plants, which in turn was associated with the replacement of natural fibers with synthetic ones and the increase in drug risk. In the last two decades, most countries have legalized the industrial production of hemp, prompting more research on this species. In Poland, a huge change in this respect was brought about by the introduced reform of May 2022, thanks to which the cultivation of industrial hemp for scientific purposes no longer requires the consent of the Chief Pharmaceutical Inspector.

Industrial hemp is an annual plant, which can be cultivated on poor-quality land, while the yield of industrial hemp has the potential for multi-directional use. The plant contains over 400 bioactive ingredients, but the main ones are cannabinoids and terpenes. Terpenes are characterized by significant anti-inflammatory and anti-cancer. Cannabidiol (CBD) shows functionality in relieving pain and promoting relaxation, while not being a psychoactive substance. Today, it is observed interest in the production of essential oils from hemp. Essential oil is a mixture of several dozen chemical components with different mechanisms of action. The quantitative and qualitative content of the essential oil depends on the hemp variety, climate characteristics and harvest time. The terpene content itself varies with specific environmental conditions, increasing with light exposure and decreasing with soil fertility. However, the production of industrial hemp bioproducts requires an efficient source of hemp raw material with appropriate quality.

The aim of the project is to find an answer to the question: how does the terpene composition of the above-ground organs change during vegetation and development in field conditions of five varieties of industrial hemp? In addition, determination of the difference in the final content of CBD and CBG (cannabigerol), essential oil and terpenes obtained from industrial hemp grown for the main inflorescence (earlier harvest) and for seeds (later harvest). The research plan includes: establishing the field experiment and cultivation of industrial hemp; determination of morphological features, plant density and biomass yield and sampling on plots; laboratory analyses; and analysis and development of results, including statistical analyses.

The field experiment will be carried out on experimental plots at the Didactic and Research Station Tomaszkowo belonging to the University of Warmia and Mazury in Olsztyn. The experiment will consist of 30 plots. The developmental/growth stages of hemp plants will be determined by observation according to the codified development phases/growth stages. Each sampling will consist of a specific mass of main inflorescences and leaves (always the same pair). The following will be determined in the laboratory: moisture and dry matter of collected plant organs, terpene composition of leaves and inflorescences, essential oil content in inflorescences, terpene composition of the essential oil, infrared spectrum of the essential oil and content of cannabinoids in inflorescences. All collected data and results will be analyzed and processed. Statistical analyzes will also be performed. This research is needed because critically, there is a lack of full understanding of the changes in the chemical composition in the development/growth stages of industrial hemp, especially when we focus on terpenes and their derivatives. There is a need to improve of knowledge of the phytochemical composition of industrial hemp, associating it with the variety, plant organ and development phase/growth stage.

As a result of the conducted research, changes in the share of terpenes in inflorescences and leaves of five different hemp varieties will be presented, depending on the stage of growth/development. In addition, it will be determined whether and how the content of essential oil, CBD and CBG varies depending on the variety and growth stage/development phase. It will be checked whether the content of essential oil depends on the content of CBD and CBG and *vice versa*. In addition, the yield of essential oil and selected compounds will be shown per 100 g of inflorescence, per single plant and per m² of cultivation area (for example, CBD or CBG yield from 100 g of hemp inflorescence, m² of cultivation area or single plant). Thanks to statistical analyses, it will be checked whether the obtained results are statistically significant, and thus whether they are not accidental.

The implementation of these studies will also add space in researches on the size of the yield of phytochemicals depending on the harvest date (e.g. linking to changes in the share of terpenes in the development cycle/growth stages) and on the energy balance of industrial hemp cultivation (linking to various stages of development from this experiment, which correspond to the specific terpene profile and harvesting possibilities on these dates).