CZECH UNIVERZITY OF LIFE SCIENCES PRAGUE

FACULTY OF TROPICAL AGRISCIENCES

Homemade Medical Hemp Products

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Declaration

I, Jan Sloup, declare that this thesis, submitted in partial fulfilment of requirements for the master degree, at Faculty of Tropical AgriSciences of the Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced or acknowledged.

In Prague, April 24, 2015

Jan Sloup

Abstrakt

Diplomová práce testuje několik způsobů pěstování léčebného konopí na pozemku České zemědělské univerzity v Praze za účelem domácího medicinálního využití. Rostliny byly pěstovány na poli, ve skleníku a uvnitř pod umělým osvětlením v hydroponickém systému. Jako jeden z předpokladů byl testován vliv způsobu pěstování na výnos usušených kvetoucích nebo plodonosných vrcholíků rostliny a na jejich obsah kanabinoidů. Hodnotilo se za období jednoho roku celkem 65 rostlin (2 ve skleníku, 3 na poli a 60 v uměle vytvořených vnitřních podmínkách – tzv. „indoor“) s celkovým průměrem 580 gramů suchých palic na 1m² indoor, 464 gramů na jednu rostlinu ve skleníku a 371 gram na jednu rostlinu na poli. Rostliny pěstované ve skleníku měly vyšší obsah THC (14,49%), než rostliny stejné variety (*„The Doctor“)* pěstované indoor (13,20%). Nejvyšší naměřený obsah THC (14,65%) byl naměřen u odrůdy *„The Great White Shark“* pěstované indoor. Výsledky této studie poukazují na skutečnost, že k produkci materiálu na výrobu léčivých konopných produktů není potřeba vysokých nákladů, které se pojí s indoor pěstováním, protože kvalitního výnosu a zároveň i dostatečného obsahu kanabinoidů pro domácí výrobu léčivých produktů lze dosáhnout i v běžně dostupných podmínkách skleníku.

Klíčová slova: marihuana, kanabidiol (CBD), hydroponie, konopná mast

Abstract

The diploma thesis focuses and tests several ways of hemp cultivation on the property of the Czech University of Life Sciences in Prague for further production of homemade medicinal hemp products. The plants were cultivated on the field, in the greenhouse and indoor under artificial light in hydroponic system. The conditions of growth have been tested as one of the prerequisites for the influence on the yield and content of cannabinoids. The evaluation has been done in one year with the total of 65 plants (2 in greenhouse, 3 in the field and 60 indoor cultivation) with total average of 580 grams of dry female buds per one m² indoor, 464 grams/ plant in the greenhouse and 371 grams/plant in outdoor conditions. The plants which were cultivated in the greenhouse had higher content of THC (14.49%) than plants of the same variety („The Doctor“) cultivated indoor (13.20%). However, our highest content of THC (14.65%) was recorded in the variety *„The Great White Shark“* cultivated indoor. The results of this study refers that there is no need for big initial investments for cultivation and subsequent production of proper homemade medicinal hemp products as the greenhouse cultivation provide comparable quality and quantity of production at lower costs.

Key words: marijuana, cannabidiol (CBD), hydroponic, hemp ointment

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1 INTRODUCTION

This thesis focuses on the problems of cultivation (outdoor – both on the field and in the greenhouse – and indoor) and production of hemp products in the Czech Republic. Project is done on the property of the Czech University of Life Sciences Prague. Cultivar called "*The Doctor*" (mostly *indica*) is chosen to be the one which is lately chosen for all conditions (outdoor, greenhouse and indoor). Yields and contents of cannabinoids will be then compared in each of these conditions. Moreover, the operational costs, time and physical labour will be also compared. For comparison, cultivar called "*The Great White Shark*" (also mostly *indica*) was chosen for indoor cultivation to evaluate the performance of another cultivar in the same conditions. Plants from all growths (indoor, field, greenhouse) received the same type of fertilizers for accurate comparison of the yields and contents of cannabinoids.

This research was carried out because of an increasing number of people are interested in home cultivation of hemp and also because of impressive health benefits of hemp ointment. It is used to cure various skin problems and irritations such as psoriasis, eczema and other dry-skin ailments. It has been reported to have anti-inflammatory properties.

This project was also done in order to find the most effective method of hemp cultivation in the Czech Republic and to give the insight into these methods of cultivation with a special focus on its economic demands.

2 BIBLIOGRAPHIC RESEARCH

2.1 Hemp: general description

Order: Rosales

Family: Cannabaceae

Genus: *Cannabis*

Common name: hemp

Hemp is an annual, dioecious, flowering herb with high adaptability to diverse climatic condition. It can be found in mild, subtropical and tropical parts of Asia, Europa, Africa, Americas and Oceania but also in cool parts such as Scandinavia or Siberia. The plant is characterized by green colour of all plant parts and specific inflorescence which produce aromatic substances and resins. The leaves are palmately compound or digitate, with serrate leaflets. The root system consists of one main root and several lateral roots which can be up to half of meter under the ground. Structure of the plant is the same like in the roots where is one main stem with several lateral branches. Leaves are changing during the process of ripening. The place of the origin has high influence on the plant habit. Also the content of the resin in the female flowers (especially delta-9-tetrahydrocannabinol - Δ9-THC and cannabidiol – CBD) is dependent not only on the variety but also on the place of growth and climate during vegetation (temperature, humidity and amount of sunlight). The contained resin (with Δ9-THC and other cannabinoids) serves to the plant as a protection against UV-light. That is why there are opinions that the hemp variety from the mild area is considered as the same variety as the one from tropical parts but with different reactions on the vegetative and climatic conditions. It is believed that there exists just one botanical genus and that is *Cannabis sativa* L. On the basis of research done on the plants in their former origin we nowadays distinguish three main species of *Cannabis* genus. Those are *Cannabis sativa, Cannabis indica* and *Cannabis rudenalis* (Hazekamp et al., 2012; Hillig, 2004; Hillig, 2005; Schultes et al., 2001).

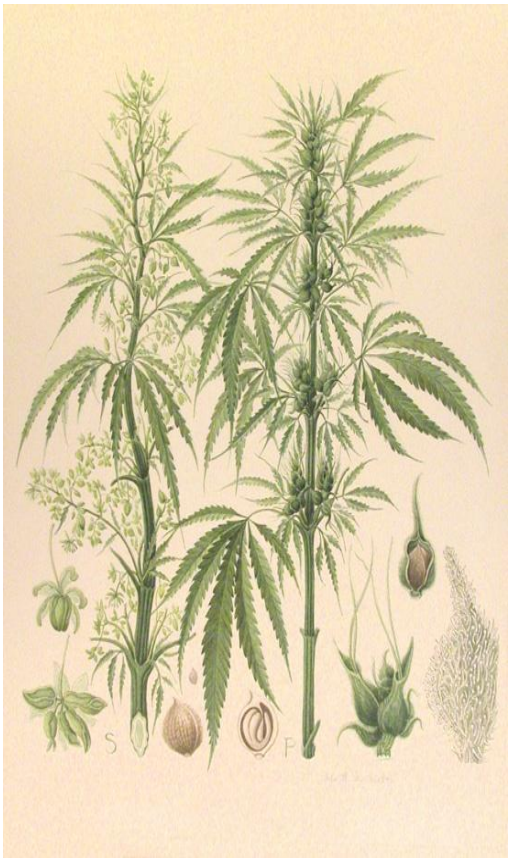

Figure 1: Herbal draw of male (left) and female (right) hemp plant

2.1.1 Cannabis sativa

Cannabis sativa is a relatively tall plant which can grow between 1.5-5.5 meters. It has not that high density of leaves and buds as *Cannabis indica* so the light can shine through them up to the ground. Its seeds are smooth without marble and dots. The development of the flowers is slow and hard to influence with the length of sunlight and light cycles. Plant originates in the areas to the south of 30° of northern latitude in the countries like Mexico, Columbia, Thailand, India or Nigeria. Content of Δ9-THC is higher in comparison to CBD. Many technical and agricultural varieties were created from this genus (mainly for fibre production in the past) (Hillig, 2004).

2.1.2 *Cannabis indica*

Cannabis indica is smaller in the comparison with *C. sativa* (between 1.2-2.5 meters). Its leaves and buds are quite dense and their colour is darker green than in the C. *indica*.

Seeds are smooth with marble. Flowering period is shorter (between 50-70 days). Its origin is in the areas around 30° of northern latitude in the countries like Morocco, Afghanistan, Pakistan, Lebanon or even Nepal. Its flowers are harder, dense and have higher content of resin with cannabinoids (Δ9 - THC and CBD). Content of CBD is slightly higher in comparison to Δ9-THC. Varieties which are bred from this species are considered as appropriate ones for medicinal purposes. They are usually used as depressant or sedative.

2.1.3 Cannabis rudenalis

Cannabis rudenalis is a weedy specie of *C. sativa* which gone wild some time ago. It grows uncultivated in Russian tundra and taiga. It is a quite small plant (30-80 cm) with thin branched stem and sparse leaves. This specie usually grows in areas above 30° of northern latitude. Flowering of this specie starts sooner and is very fast. That is because of its adaptation on early winter conditions. This specie is also more resistant to pests and diseases thanks to its wild adaptations. Content of cannabinoids is low (CBD a little bit higher than Δ9-THC). Varieties or hybrids which are bred from this species are not that dependent on sunlight or photoperiod. Created hybrids are known as *autoflowerings*. It basically means that they are ripe after specific number of days (usually around 70) without regard to the length of sunlight. Good example is a hybrid between *C. sativa* and *C. rudenalis* which can get fully ripe in the areas with mild climate.

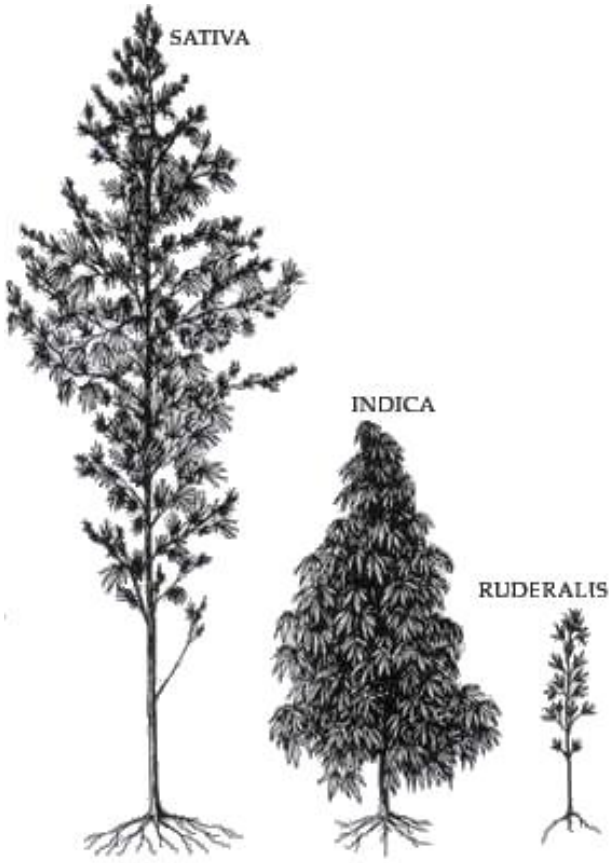

Figure 2: Visual difference between individual Cannabis species (*sativa*, *indica* and *rudenalis*)

2.1.4 Hybrids

Nowadays there are many cultivars which were created by crossbreeding of the three existing species. Those created cultivars have different attributes according to purposes for which they were made to. Breeding of *Cannabis* species focuses mainly on early ripening, duration of the flowering, yield, psychotropic and medicinal effect or resistance against pests and diseases.

Hybrids were created from different genetic cultivars and are generally more resistant than the pure species (thanks to heterosis). They are usually the best for growing in artificial environment (indoor) because their choosing and breeding is mostly done in these places. They can be also easily placed and grown outside (outdoor) because they can ripe before the winter conditions. Hybrid production can provide combination of interesting cultivars with many enhanced attributes (Hillig, 2004).

2.2 Reproductive cycle of Hemp

Hemp is a dioecious plant which means that there are female plants and the male plants or even hermaphrodites or androgenic ones. Only female plants are used for production of medicinal products because the content of cannabinoids is higher than in male plants. It is necessary to get rid of all male plants before the pollination because the female plants are then depreciated by seed creation which therefore leads to lesser content of cannabinoids. The pollen can be displaced on very long distances and that can therefore lead to difficulties when two fields are in reachable distance from each other (Edward et al., 1992; Moliterni et al., 2004).

2.2.1 Male plant

Adult male plant creates flowers which can be found all over the male plant. The male flowers produce very light pollen which sticks to the pistils of the female plant which is afterwards pollinated. It is almost impossible to distinguish the male plant and the female plant during the vegetative period but fortunately the male flowers ripe earlier than the female ones. Male flowers flower about 7-10 days earlier than the female ones of the same variety. Male plants are usually grown only for breeding purposes due to the lower content of cannabinoids in the plant (Edward et al., 1992; Rothschild et al., 2005).

2.2.2 Female plant

Adult female are creating grape-like flowers which are formed of small buds and pistils. Every bud is containing an egg which can be fertile after contact with the pollen. Male plants produce pollen grains which stick to the long pistils and then go down to the buds. The seed is created in the bud after successful pollination. The seed is always a product of both plants. If the parents are picked from the pure line or from stabilized hybrids their seeds will be then with very strong (almost 100%) genetic basis. Almost all of the seedbanks in the world are offering hybrids instead of pure cultivars because the hybrids are usually stronger and have more useful properties (higher yield, resistance against cold or diseases, shorter photoperiodism ect.) and because the pure *C. indica* or *C. sativa* is

nowadays almost impossible to reach due to the leakage of genetic material in the sixties (Edward et al., 1992; Moliterni et al., 2004; Rothschild et al., 2005).

2.2.3 Hermaphrodite plants

Genetic mutations can lead to creation of the plants which have female flowers as well as male flowers. Those plants are called bisexual or hermaphrodite. There are plants with more female flowers than male flowers as well as the plants which have more male flowers than female flowers. It means that the level of hermaphrodite can be different in different plants. But hermaphrodites are not created just in the lab. They can be also found in nature but more often in the indoor cultivations. It has been found that the plant can turn into hermaphrodite when exposed to some extreme conditions or just because of some external stress (high or low temperature, lack of water, overfertilization or irregular photoperiodism). This happens usually in the indoor cultivations because the switching between warm and cold or day and night is much more gradual in the nature than in the indoor conditions (Edward et al., 1992; Moliterni et al., 2004; Rothschild et al., 2005).

2.3 Photoperiodism

Photoperiodism or light cycle is one of the key factors which influence the cultivation and also the flowering of *Cannabis* species. *Hemp* plants are following the changing of the four seasons in the nature and are adapted to those changes (except the areas where is winter too cold and mild areas where the cultivation is possible only during late spring, summer and beginning of autumn). Plant is not reacting only on the change of the photoperiod but also on the changes of temperature or humidity. But the light remains the most important factor for the changing of the vegetative period into the flowering period. Generally accepted length of light for the vegetative period is 18 hours of light and 6 hours of dark per day (Lisson et *al*., 2000). On the other hand the generally accepted length of light for the flowering period is 12 hours of light and 12 hours of dark per day. It basically means that if you cultivate outdoor you are depending on the sun for

the starting of the flowering period but if you cultivate indoor you can start the flowering period whenever you want. Usually when the plants are grown enough and ready for the flowering part (Cosentino et al., 2012; Lisson et al., 2000).

2.4 Outdoor cultivation

One of the most important things is that the length of outdoor cultivation is different according to the latitude and place of cultivation. The latitude influences the season and photoperiodism. In the case of outdoor cultivation hemp can be grown in two climatic regions:

- First region is located between 0° and 30° of both southern and northern latitude where the cultivation is possible for whole year. One of the most popular mostly *sativa* cultivars come from this regions (Colombia, Mexico or Thailand).
- Second region is located between 30° and 60° of both latitudes where the cultivation is possible only during a part of the year. Morocco, Lebanon, Turkey, Pakistan or Afghanistan are representative countries of mostly *indica* cultivars in this region. Production of hashish is very popular in these countries. Europe with USA and Canada are considered as areas belonging to this region.

As it was mentioned in the previous chapter, photoperiodism is a difference between the length of the day and length of the night. Hemp plants are starting to bloom thanks to this process when the nights become longer (Cosentino et al., 2012; Lisson et al., 2000). There are no big differences in photoperiodism when you are close to the equator but the changes are bigger and bigger with increasing distance from the equator and also the weather is not so stable (higher differences in temperatures during the day and during the night).

In the Czech Republic (mild climatic zone) the days become longer after 20th of March. Temperatures are getting higher in the April and therefore the seeds are starting to germinate. Then the plants are creating their structures with leaves and branches. After the 21th of June the days are becoming shorter again and that is a signal for the plant to start investing energy for creation of flowers. The plants are fully turned to their

flowering period approximately at the end of August (nights are long enough for the plants to start flowering). For summary of the outdoor cultivation the plants are starting to grow at the end of April (or beginning of May) when the night frosts are gone. Thanks to this they have enough time for proper growing before they start to produce flowers. Harvest is usually done at the end September or in the October (Cosentino et al., 2012).

Figure 3: Intercropping of hemp with other ornamental and medicinal plants

2.5 Indoor cultivation

In the case of indoor cultivation it is necessary to effectively replace natural elements for successful yield and quality. Sunlight is in this case replaced by some other source of light (fluorescent lamps, discharge tubes, diodes or simple bulbs). The wind which is also very important for strong plant structure (ability to bear big and heavy flowers) is replaced by

some fan or ventilator. But in the case of substrate there are more options and therefore more methods of indoor cultivation.

First one is the most similar to the outdoor cultivation because the plants are still cultivated in flower boxes with some kind of substratum (classic soil, sandy soils or cocoa pulp).

Figure 4: Indoor cultivation with substrate and pots

On the other hand no substratum is used in the other two methods of cultivations (hydroponics and aeroponics). The water is stored in the reservoir under the system, the water pump is placed inside and supports with water the plants above. The water then flows through the overflow back to the main reservoir. The plants have very fast intake of nutrients because of permanent intake of water and therefore are ripen a bit faster. This method is considered as a little bit more difficult than in the case of substrate cultivation because overdosing with fertilizers is in the case of hydroponic cultivation very fast and

fatal (Decorte, 2010). Many hydroponic systems are nowadays available in many online or even brick stores and considered as a little bit more expensive (in comparison with classic substratum cultivation with pots.

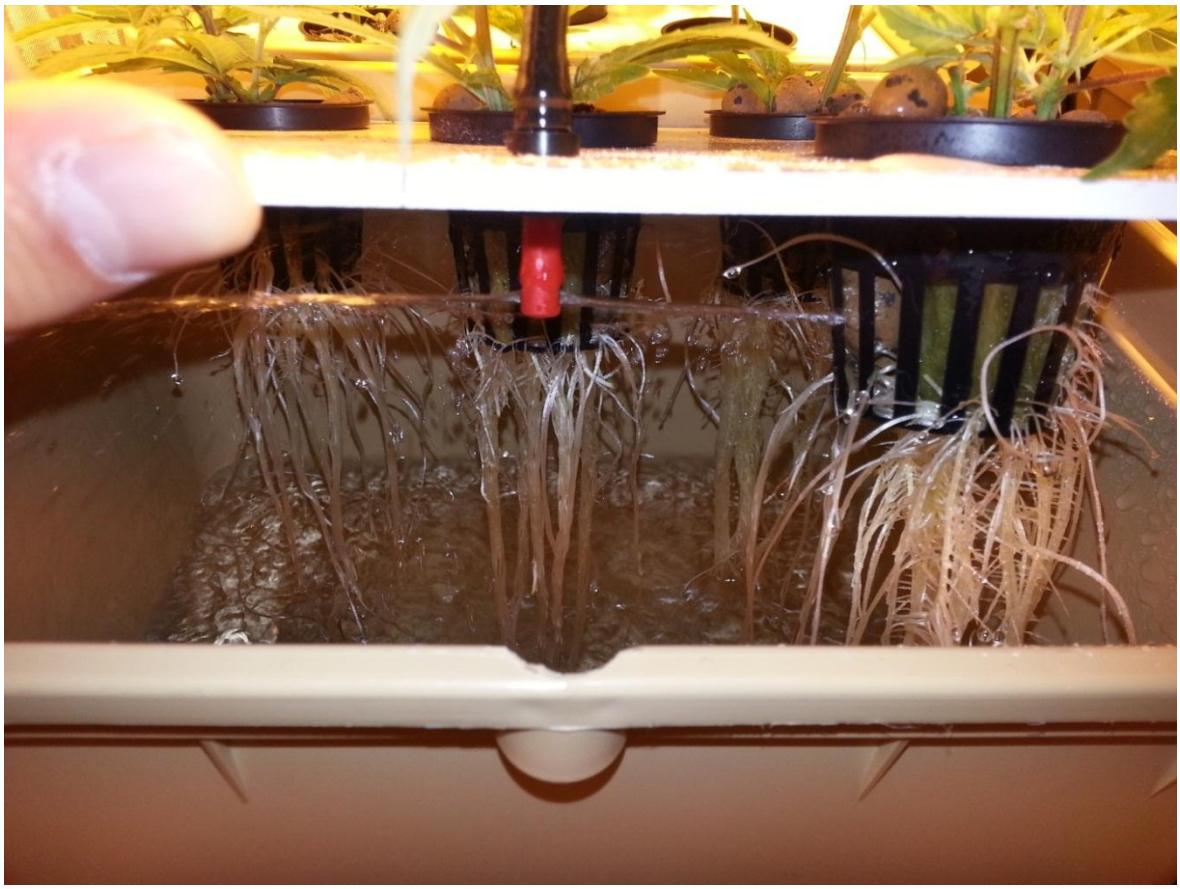

Figure 5: Indoor hydroponic cultivation (Dutch pot system) with detail of the irrigated roots

Aeroponic cultivation is very similar to the hydroponic but with one exception and that is that in the case of aeroponic cultivation the plants are permanently supported with water but in the gaseous state (vapour). There is again the main reservoir with water pump but there is also special nozzle located on the end of the supporting pipe which provides 100% humidity on the roots of the plants. The water is then condensing on the roots and then fall in drops back to the main reservoir. This method is also considered as a little bit difficult and expensive (Szabonagy et al., 1994).

Other important aspects which are necessary for successful indoor cultivation and good yield are cultivation place (room, wardrobe or tent which is non-translucent), light shield (for better light rebound) and stable temperature around 20 °C (Toonen et al., 2006).

2.6 Vegetative period

When the plant becomes deeply rooted in the soil, hydro or aero system, it starts to grow and develops strong structure which is necessary for later flowering period. The plant starts to develop new branches and have higher requirements for water, light, CO_2 and fertilizers (especially the nitrogen). The vegetative period is very crucial for the right course of the flowering part. When the vegetative part goes well the plant will get big deeply green branches and leaves which fulfil the plant with energy and serve as a strong structure that is necessary for having big and strong flowers later.

The light is just as important as CO_2, temperature and amount of fertilizers. The more of those nutrients the plant can absorb the more developed the plant can be. The soil gets dry faster with the development of the plant and the irrigation more demanding. If some of the lower leaves start to turn into yellow during vegetative period it is a sign that the plant needs more fertilizers for growing.

The length of the vegetation period depends on the type of cultivation. If hemp is cultivated outside (garden or greenhouse) it relies on the sun and the photoperiodism which is in the specific area (in the Czech Republic the end of vegetative period and beginning of the flowering period occurs usually at the end of August).Generally the length of the vegetative period for outdoor cultivation can be 1-4 month (depends on the area where you have the plants). For indoor cultivation can the length of the vegetative period be adjusted as long or as short as needed. The light can be reset (which replaced the sun in this case) from 18 hours of light a day to 12 hours of light a day and that is everything. It is very subjective – and therefore it is the weak point of indoor hemp cultivation – to determine the right time when the plant is strong enough and ready to start flowering. The suitable vegetative period for indoor cultivation is between 1-35 days where the range depends on how big plants are expected (Knight et al., 2010; Lisson et al*.,* 2000; Ross et al., 2005; Toonen et al., 2006; Vanhove et al., 2011; Vanhove et al., 2012).

Figure 6: Indoor hydroponic cultivation with fresh cuttings of „*The Doctor*“ variety

2.7 Flowering period

The moment when the light cycle is changed from 18 hours to 12 hours of light a day is not yet the beginning of a real flowering. In the first two weeks the plant still grows and makes structures for the flowers. It grows to the high and the distance between individual levels of branches is reducing. These first 14 days of flowering period mostly refers to the vegetative period. The plant should receive fertilizers which are given for the vegetative part in the first 14 days (no phosphorus or potassium). The plant still grows and needs mostly nitrogen in the first part of flowering period. The pistils can show up after three or four days but the real flowering begins when there are many of them. When the structure is stabilized (10-14 days) we should pay attention to the size and height of the plants. All flowers located in the lower part of the plant should be removed to provide the best nutrition for the flowers which are located in the upper part and lit by the sun or the bulb.

The flowers which grow in the shadow don't create compact buds and their number of leaves is higher than the number of calyxes. When the flowers are properly lit they create big grape-like structures with minimal number of leaves. The leaves are never to be cut off because the plant cannot develop very well without them. Usual mistake is cutting off the big leaves which make shadow on the flowers. The size and compactness of the bud is determined by the amount of light which drops down on the specific leaf which is connected with its bud. The fertilizers for flowering shall not be used unless the flowers are developed on the plant. When the flowers start to develop the development of the leaves is almost over. Pistils are white and growing in size very fast. Leaves which are near to the flowers are covered with fine white resin. This is a proper time to use fertilizers determined for flowering period (phosphorus and potassium).

Temperatures ranging between 25 °C (day or light period) and 15 °C (night or dark period) are ideal for the plants and are also instrumental in preventing against expansion of the harmful microorganisms and insects (*Tetrachynus urticae, Heliothrips haemorrhoidalis, Trialeurodes vaporariorum or Hemiptera* spp.).

Flowers are getting bigger, closer to each other and are heavier. The branches may start to bend under the weight of the flowers. The plants can be fixed with the net or just tight to some static support to prevent bending and therefore breaking the branches. It is necessary to maintain the humidity below 50% when the flowers get close to each other. Otherwise we risk spreading of the fungal diseases *(Botrytis cinerea, Fusarium oxysporum or Plasmopara viticola*) in the buds and therefore devaluation of the products. The flowers are now becoming bigger and make compact buds.

At this time of flowering the smell of the plants starts to become very strong and intensive. In the case of cultivation in the soil, rockwool or cocoa fibres the fertilization of the plants should be stopped approximately 10-14 days before the harvest to make sure that all of the minerals and rests of the fertilizers are flooded out of the plant. The plant starts to use minerals and fertilizers contained inside of it in the case of lack of those from the outer environment. Substrate should be flooded with clean water.

When the plant starts to turn yellow it is a sign that the plant had lack of nutrition and has started to use chlorophyll instead of it. The plant starts to use chlorophyll for making energy in the case of lack of minerals and other nutrition. This is the right time for harvest (Consetino et al., 2012; Knight et al., 2010; Lisson et al., 2000; Lisson et al., 2000; Potter et al., 2008; Ross et al., 2005; Toonen et al., 2006; Vanhove et al., 2011; Vanhove et al., 2012).

Figure 7: Indoor hydroponic cultivation with „*The Doctor*" variety (early blooming phase)

2.8 Harvest

To determine the perfect moment for harvesting is rather complicated. It can be said that it is similar to grapes. Means the more late harvest the more sugar will be contained and furthermore the more alcohol can be made of it. The taste and the effect will be lighter if you harvest hemp sooner than in plants which were harvested at right time. On the other

hand the taste and effect could be really too strong or can become blunt if you harvest hemp too late. Canadians have very good experiences with indoor cultivation and they prefer the harvest right before the pistils turn red or brown. On the other hand the Dutch and Americans prefer harvesting when two thirds of pistils are already red or brownish. It can be said that it all matters on the taste and needs of the grower. In general if you leave the plant more time for ripening the harvest will be bigger but the quality will lose something. If you seek for the stimulating effect you have to harvest the plants partially not ripen (50% of pistils turned red) and if you seek for slightly blunt effect you have to harvest fully ripe plants. Although the pistils are very good indicators of ripening and can serve very well to almost all growers the best and the most precise indicator is the resin. If you take a look on the resin through the magnifying glass you can see enlarged glands. At the beginning they are slightly deformed and transparent but during the ripening they round off and get amber colour. When the most of the resin is round off, shiny but still transparent then the plant is ripened. After that the small marbles of the resin react with oxygen and get amber colour. The quality of the resin goes then slightly down (Toonen et al*.,* 2006; Vanhove et al*.,* 2012).

Figure 8: Indoor hydroponic cultivation of „*The Doctor*" variety (late blooming phase)

2.9 Postharvest handling

The plants need to be dried after the harvest. The process of drying is very essential and has high influence on final taste of the product. Basic principle is to leave the plant alive after the harvest as long as possible to make sure that all the chlorophyll has changed into saccharides. Right after the harvest the plant is full of chlorophyll which is very bitter. Active substances have not yet changed into Δ9-THC. THC is present but it takes a few days of drying until it is fully active. The plant should be placed in some dark place to stop the process of photosynthesis and process of transpiration. It is better to dry the plant upside down because then it has the feeling that it is still alive and therefore the plant is consuming the rests of its chlorophyll because it has no other energy or nutrition. Temperature should not overcome 17-18 °C and humidity should not be higher than 60% otherwise the drying plants can suffer from fungal diseases (Knight et al*.,* 2010; Vanhove et al*.,* 2011; Vanhove et al*.,* 2012).

2.10 Cannabinoids, endocannabinoids and their receptors

Cannabinoids are substances which occur in nature exclusively in hemp plants. The system of its usage by living organisms is called cannabinoid system. Plant cannabinoids in the human body act the same way as the endocannabinoids (own body substances). Cannabinoids and endocannabinoids are bind with special connectable places called the cannabinoid receptors (CB receptors). Cannabinoid system consists of several protein receptors located in human (even animal) body (Adams et al., 1996; Martin et al., 1999; Scott et al., 2004; Williamson et al., 2000). These receptors are occupied by effective substances which are divided in two categories. First are endocannabinoids which human body can actually produce and the others which are produced by green parts of hemp plants (fytocannabinoids or just cannabinoids).

2.10.1 Cannabinoid receptors

Nowadays, the well-known receptors are CB1 (cannabinoid receptor type 1), CB2 (cannabinoid receptor type 2), PPAR gama (gama receptor) and TRPV1 (vanilloid

receptor). CB1 receptors are found in brains (specifically in the cerebral cortex), hippocampus and in other brain parts such as in spinal cord and in whole central nervous system (Ameri et al., 1999; Cabral et al*.,* 2008; Felder et al., 1998). Moreover we can find them also in endocrine system, salivary glands, white blood cells, spleen, heart, lungs, skin, bones, reproduction system, urinary system and in digestive system. CB2 receptors are found in cells of immune system (white cells and leukocytes), tonsils, spleen, thymus gland, pancreas and in bone marrow. PPAR gama receptors are found primarily in the fatty tissues but also in some brain parts. Vanilloid receptors refer to basal ganglia and nerve endings. The number and position of receptors and endocannabinoids in the body is changing in case of illness. For example in the case of pain our body starts to flood brain parts responsible for pain with anandamin which helps with reducing the pain. Flooding of anandamin to the intestine in case of the lack of food is good for stimulation of our sense of taste. The number of receptors is higher in the case of chronic nerve illnesses or inflammation (Cabral et al*.,* 2008; Felder et al., 1998; Munro et al*.,* 1993; Pertwee, 2008; Schatz et al*.,* 1997).

Currently, the known endocannabinoids are anandamid (N-arachidonoyletanolamin, AEA), 2-arachydonoylglycerol (2-AG), 2-arachinodyl glyceryl éter (noladin éter), O-arachidonoyl-etanolamin (virodhamin) or N-arachidonoyl-dopamin (NADA). There are more than fifty other derivates in the human body which can act as endocannabinoids and are able to activate or block cannabinoid receptors (Freund et al., 2003; Martin et al*.,* 1999; Wilson et al*.,* 2002).

2.10.2 Fytocannabinoids (cannabinoids)

Nowadays we can distinguish up to sixty six fytocannabinoids, where these substances can exist in mutual interaction and a lot of them are just derivate of previous substance. They are divided according to this on ten types: Delta-9-Tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), Cannabinol (CBN), Cannaabigerol (CBG), Cannabichromen (CBC), Delta-8-THC, Cannabicyclol (CBL), Cannabielsoin (CBE), Cannabinodiol (CBND) and Cannabitriol

(CBTL). We can assign several cannabinoids to each of these types which were mentioned. For example in the case of Δ9-THC, we can distinguish nine cannabinoids.

The number and the concentration of cannabinoids vary with different varieties of hemp plants. The most important ones are Δ9-THC and CBD. Hemp plants can be easily divided according to the content and concentration of those two cannabinoids. Plants with medium to high concentration of THC and CBD are varieties from tropical and subtropical areas of Asia and Africa. Plants with high content of THC and low content of CBD are varieties originated in tropical areas. On the other hand the plants with low THC content and high CBD content refer to agricultural varieties. And the last type with low THC and CBD content belong to the technical varieties (Grotenhermen, 2003; Hillig et al., 2005; Russo et al*.,* 2006; Williamson et al*.,* 2000).

The ratio of cannabinoids is also very important and it determines the suitability of the plant for different illnesses. For example most of the patients recommend varieties with equal but high concentration of THC and CBD which guarantee complex effect during reduced psychoactivity.

Endocannabinoids and cannabinoids have different chemical composition but they work very similar. In human body, the endocannabinoids provide regulation of energetic homeostasis (balance of organism), metabolism, it also supports immune system, influence the sense of taste (regulates the food intake), influence the sense of pain, movement coordination and last but not least the memory. Furthermore they help in struggle with stress, anxiety and are responsible for its adaptable reactions (Martin et al., 1999; Wilson et al*.,* 2002).

Cannabinoids which are bind with CB1 receptors work analgesic (against pain), antiemetical (against convulsions and vomiting), hypothermical (reducing the body temperature) and against hypoactivity. Neuroprotective effect of anandamid and THC is very important during autoimmunity illnesses, cancer or in regulation of blood pressure. Furthermore long- term intake of cannabinoids supports the creation of new brain cells, helps with getting rid of stress, suspense, anxiety and works against depressions. CB1 receptors in brain are fully responsible for psychoactive effect of cannabinoids.

Cannabinoids which are stimulated by CB2 receptors in low portions supports the activity of immune system. In higher dosage they work against and soften the activity of the immune system. In general they have anti-inflammatory effect (Ameri, 1999; Burns et al., 2006; Croxford, 2003; Grotenhermen et al., 2012; Hampson et al., 1988; Lastres-Becker et al., 2005; Lynch et al., 2011; Rahn et al., 2009; Russo et al., 2006; Scott et al., 2004; Williamson et al., 2000; Zajicek et al., 2003; Zuardi et al., 2006).

2.11 Hemp products and their usage

There are many ways how to use hemp and its appropriate way of use and dosage depends on the illness, variety of the plant, method of cultivation, method of harvest and postharvest handling (Borgelt et al., 2013; Potter et al., 2008; Ross et al., 2005).

2.11.1 Hemp ointment

Probably the most common way of hemp medicinal use in the Czech Republic (except smoking) is making and greasing the hemp ointment. It is also considered as one of the easiest ways of usage. The *hemp* ointment is very often used against great variety of skin diseases (such as psoriasis, eczema, herpes, pimples or rashes), injury (abrasions, burns or cut), joint-ache, muscular-ache or bedsores. Homemade hemp ointments (they are usually based on common fat or medicinal Vaseline but sometimes even on Shea butter, coconut oil or bee wax) can easily compete with official cosmetic products which you can buy in stores. Hemp ointments are also favoured because of their good effectiveness and also because it is possible to use lesser potent parts of the plant for ointment creation (male flowers, seeds or leaves). One of the disadvantages is that the cannabinoids have lower absorb through the skin. That is why hemp ointment is usually usable only for local but not complex injury. It is possible to feel stupefy In the case of strong hemp ointment (high concentration of THC) but only after application into places where the big arteries are located (for example on temples).

For homemade production of the ointment white medical Vaseline is needed (because it has the highest durability) and a plant material (leaves, flowers ect.). Approximately 10 grams of dry flowers is good enough for 200 grams of medical Vaseline. Plant material should be coarsely crushed (not finely otherwise a lot of plant material will remain in the ointment). After making water bath, the Vaseline is put inside and until melted and then plant material is add. The mixture is boiled approximately for two hours and then left to cool down in some dark, dry place with room temperature. Approximately after five days the whole procedure is repeated. Small amount of plant material can be added with every additional repeating of the procedure. The whole process should be repeated at least 3 times. After the last procedure, the mixture is filtered through some linen cloth to the bowls and left it to become stiffer. The rests of the plant material which remained in the linen cloth can be stored (frozen), because they are considered as a very good against burnings, sting bites or abrasions (Arjan Roskam; Joachim Helms; Franco Loja, personal communication, November 2014).

2.11.2 Hemp cooking

Another popular type of hemp usage is cooking with hemp. It is usually used when long-term supply of cannabinoids is needed (Parkinson´s disease, Alzheimer ´s disease, epilepsy or arthritis. It can be used as a simple spice (milled female flowers or leaves) with any fatty or oily food. The disadvantage of oral application is its lower biological disposability (5-10%). That is why it is recommended to use decarboxylation of the hemp before cooking (for example to preheat the plant parts in the oven). Another disadvantage of oral application is that the effect has slower kick-in (30-90 minutes) and therefore it increases the risk of overdosing.

Hemp products are usually not very palatable (because of the chlorophyll) and thereafter it is very hard to digest for our digestive system and therefore it has bad influence on livers. It is generally better to extract THC in lipids and after that use it in the kitchen. The rests which have been left after hashish production (100-150g of leaves and flowers on 500g of butter) or there can be used unused small flowers (50g of flowers on 500g of

butter). Generally the butter which was made with the rests from hashish has a better taste because the chlorophyll (which is very bitter) is there in lesser concentration. The butter must be melted on mild fire without stirring. When the butter has melted down the foam which has occurred on the melted butter surface (casein) needs to be removed. Then put the butter to big cooking pot with the plant material and add a bit of water. The mixture needs to be permanently stirred and heated (without boiling). Then use the sieve to remove as much plant material as you can and filtrate it again. Then put all the plant rests to prepared dish towel and compress it (to remove the rests of the butter from the plant material). This must be done when the plant material is hot otherwise the butter will stick to the plant material. The process can be repeated to gain maximal amount of the product. When you decide that it is ok you remove all the plant material and then put the mixture to the refrigerator to cool down. After a few hours the fat will swim out to the surface and will make a crust. The crust (final product) is removed and creates the base for further cooking with hemp (Arjan Roskam; Joachim Helms; Franco Loja, personal communication, November 2014).

2.11.3 Hemp tincture

Hemp tincture is practically an infusion of dry flowers in alcohol. Extracted droplets have higher effectiveness in correct use (short flattening in the mouth so that the mucous membrane in our mouth can easily absorb the cannabinoids). It is easy to determine the right dosage if you work with time-proven or already tested varieties. Hemp tinctures are being used when you need permanent supply of cannabinoids (anorexia, intestinal problems but also in treatment of inner inflammation or painful conditions).

2.11.4 Hemp extract

Hemp oils are made by washing off the resin from the female flowers by pure alcohol, ether or medicinal petrol and subsequent evaporation. The process of its creation can be dangerous because of the risk of explosion so it is necessary to follow correctly the instructions. The manual from Rick Simpson (who called his extract Phoenix tears) is

probably the most known one. The hemp extract is able to supply the body with cannabinoids in high concentrations. Extract can be used not even oral but also by inhalation, ointments or also in the form of vaginal or rectal suppository. In the case of oral use the small droplet of extract is placed under the tongue or is spread on the gums where it is successively absorbed (Brady et al., 2004; Hosking et al., 2008; Notcutt et al., 2004; Russo et al., 2007).

The biological disposability of effective substances is high in this case but not everybody is able to withstand this usage because the extract has slightly bitter and spicy taste. Extract can be then dissolved in the oil, fat or in some beverage which people find tasty.

Inhalation is in many cases the first type of use of cannabinoids. It is also relatively widespread. We can divide inhalation on traditional smoking and nowadays very popular vaporization. In the process of inhalation by modern inhalation devices (vaporizers) the temperature of inhaled material can be precisely set so that the effective substances can be evaporated with water (vapour) and not burn. The mixture shall not be burned to avoid the carcinogenic product (tar or carbon dioxid) is able to reach the body. The vaporized vapour is cooled on its way through vaporizer so the lips cannot suffer from burnings or scalding. Another advantage of inhalation is that the effect comes relatively quickly (2-8 minutes) so the dosage can be set easily. It is particularly used during acute asthma attacks, various aches, convulsions, anxiety or depressions (Holdcroft et al., 2006). When compared with smoking which has high health risks the vaporization is a safe way of application which brings fast alleviation to non-smokers, elder people or even kids.

Hemp extract is becoming more and more popular. The reason for this is that the extraction with using butane is very easy. Final product has much more better quality because the butane is more selective solvent than alcohol. It basically means that it doesn’t melt down the chlorophyll and tannins. Final product is very pure oil with amber-like colour and pleasant flavor. This method allows extraction even with lower amount of plant material which is an advantage for people in the cities who cultivate only for covering their own needs.

Plant material (flowers or small leaves), butane (in the form of small gas container for refilling the lighters), plastic tube (with cap) and a bowl are all you need for proper extraction with butane. The process of extraction should be done outside and never in the room to minimize the level of danger (high concentration of butane is very explosive in the air). There should not be any fire or sparks during the extraction. Smoking is also not recommended.

First is to pick up some suitable place in the outside environment (for example garden or balcony). Then make a powder from the plant material which you want to use for the extraction (the plant material can be placed in the freezer before that). We can use leaves, flowers and almost all plant material because butane extracts just THC. The plastic tube should have appearance like a saltcellar with holes in screw cap. Coffee filter is placed under the screw cap and the tube is filled with the plant material but without pressing. The tube has small holes in its screw cap and one bigger hole on the opposite side for the butane container. The bowl is placed under the tube and the jet from the butane container is put to the hole in filled plastic tube. Then we press until the butane container is empty. Yellow liquid starts to run out through the holes in the crew cap. It is butane in the liquid form. When all liquid comes out you leave it in the room temperature and the butane will evaporate. Yellow and translucent paste will remain at the bottom of the bowl approximately after two hours. This final product has then appearance like a honey (Arjan Roskam; Joachim Helms; Franco Loja, personal communication, November 2014).

2.11.5 Hashish

Hemp plants produce the resin which works in the nature as a protection against pests, diseases and the sun. This resin (hashish) contains active substances and essential aromatic oils. The resin is contained in the bubble which is called the gland. The gland is located on the fine hair which is called the trichome. Most of the trichomes are without its glands during the vegetative period. The glands start to develop during the flowering period and only on some parts of the plant (flowers). The trichome and the gland are

connected at the beginning and the gland starts to change its colour during ripening. When the plant is fully ripe the connection between the gland and trichome is weakening until it is completely separated. The glands can now fall off with gentle shaking of the plant (Gaoni, 1971; Gaoni et al., 1964; Mechoulam et al., 1965).

There are many ways how to extract or separate hashish from the plants but the most favourite method in the Czech Republic is separation with ice and water. This method is broadened and also very effective because the dust and fungus spores are separated with this method. Two or more special sieves are needed, one container big enough for the sieves, electrical mixer or power drill and of course ice. The sieves should be different where the first one should have bigger holes inside (to let the hashish run through) and the others should have smaller holes (for catching the resin). The method of water extraction is using the physical attributes of the resin and green parts of the plant (resin sinks down and on the other hand the green parts float).

At first you put the bag with the mild sieve into the container. Then you put the other bag with coarse sieve inside of the first one. You fill the container to the half with water and add the ice. Then you put the plant material on the ice and push it into the middle. Then you mix the mixture with the mixer or power drill but very carefully to not damage the sieve bags. You leave the plant parts to sink down and you repeat the process. After this you leave the mixture for at least one hour to sit down and then you take out the first bag with the plant material. Then you take out the second bag with the wet resin (hashish) at the bottom of the sieve. You can use a spoon or the credit card is considered as the best tool for scraping off the resin from the sieve. The hashish is dry in between 24-72hours depending on the surrounding environment (Arjan Roskam; Joachim Helms; Franco Loja, personal communication, November 2014).

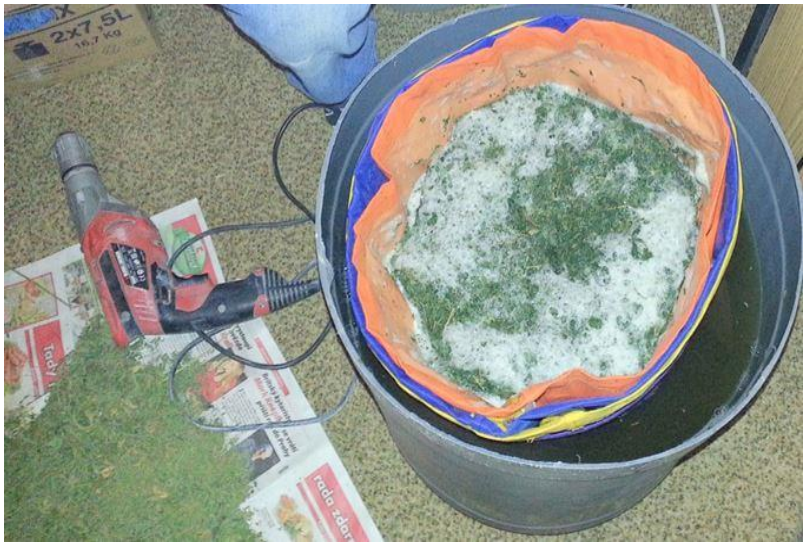

Figure 9: Hashish production by using the ice bag technique

Figure 10: Hashish collected by using the ice bag technique

2.11.6 Other Hemp products

There also more methods of cannabinoid use such as suppository, eye drops (suitable for treatment of glaucoma or eye inflammations), sprays, plasters or injections. The suppository method is probably the most favoured among the patients because of its two times faster effectiveness, easy control of dosage and good toleration during digestive problems. If you want to prepare suppository at home you just have to boil down the hemp in cocoa butter or some other fat (bee wax can be added for better consistence) and gained substance is put into prepared molds and stored in the refrigerator.

Injection application of cannabinoids is exclusively related on research purposes and on synthetic cannabinoid applications (mostly the equivalents of THC). That is because the natural cannabinoids cannot be dissolved in the water (or very hardly).

2.12 Legislation in the Czech Republic

Cultivation and using of hemp in Czech Republic is regulated by the law of addictive substance number 167/1988 and its last novel number 273/2013 from 22. August 2013 that also influenced handling with medicinal hemp. According to the law the word hemp means flowering or fruitbearing cyme of the plant from the genus hemp. Paragraph 4 provides that there needs to be permission for manipulation with addictive substances and their preparations. On the other hand paragraph 5 says that no permission is needed when cultivating and using hemp plants which have lower content of tetrahydrocannabinols than 0.3%. Cultivation and using the varieties which have higher content of tetrahydrocannabinols than 0.3% is forbidden. But from the first of January 2014 there could be even permissions for cultivation and handling with varieties which have higher content of Δ9-THC. The law does not relate to the cultivation for scientific purposes and also does not relate to cultivation in order to preserve genetic diversity of the *Cannabis* spp.

According to the paragraph 15 subsection e it is also against the law to produce or extract hemp resin and substances from the group of Δ9-THC. At the beginning of 2013 there was approved one law number 50/2013 which enables distribution of the medicinal hemp. Three other laws (378/2007 about medicine, 167/1998 about addictive substances and 634/2004 about administrative charges) needed to be changed according to the new one.

The new law respects international agreements and also makes the hemp available to the patients. Only doctors are authorized to prescribe the hemp and only for the diagnoses defined by state law. The prescription should be deposited to the central repository of the electronic prescriptions. It should then be easy to find out who has got the hemp, when and how many of it to prevent the misusing on different purposes. Medicinal hemp can come from imports or even from the internal companies which have special license for its

cultivation. The license for import and for cultivation is given by the State Institute for Drug Control (SÚKL). The first two licenses for import of hemp were given to the companies in November 2013. Shame is that the state institute for the inspection of the medicine has not categorized the medicinal hemp to the group of medicine which is covered (paid) from the medicinal insurance. This basically means that the price of medicinal hemp will be at least the same or maybe higher than in hemp which you can buy on the black market.

Overview of the basic legislation pertaining to the activity of growing hemp for medical use, its prescription and dispensing available online on the website of State Agency for Medical Cannabis (SAKL): http://www.sakl.cz/en/index/legislation [accessed on April 24, 2015].

Figure 11: Ideal harvesting bud structure of „The Doctor“ variety

3 AIMS OF THE THESIS

The aim of the thesis was to compare various types of cultivation of hemp for preparation of homemade medicinal products. One variety ("*The Doctor*") was cultivated in three different conditions: indoor, on the field and in the greenhouse in order to evaluate the economic, time and labour costs of various types of cultivation.

As the indoor cultivation of hemp is rather sophisticated process, it can be expected that the product will be of the highest quality and therefore another variety ("*The Great White Shark*") was cultivated in indoor conditions in order to proof whether there is an influence of the variety on the quality or quantity of the product.

Specific aim was to describe the prospective growing techniques and production of the hemp products in the Czech Republic.

3.1 Hypotheses

H1: The content of cannabinoids will be higher in indoor cultivation because the growing conditions (temperature, light, humidity etc.) is more stable in indoor than in outdoor growth in the Czech Republic.

H2: The content of cannabinoids will be similar in both indoor cultivations ("*The Doctor*" and *„The Great White Shark"*) because the growing conditions for these two cultivations were exactly the same.

4 METHODOLOGY

4.1 Plant material and cultivation

All Hemp plants were cultivated and harvested at the Czech University of life Sciences between May and October 2014. The plant materials (feminized seeds) were bought at Prague Cannafest in November 2013. We have chosen *"The Doctor"* (mostly *indica*) as an appropriate cultivar for our outdoor x indoor experiment and *„The Great White Shark"* (also mostly *indica*) for comparison with *"The Doctor"* in the indoor conditions. All plants were therefore cultivated from cuttings to minimize the costs of the seeds because they can be expensive. We made three mother-plants from three seeds of *"The Doctor"* and two mother-plants from two seeds of the *„The Great White Shark"* to be sure that we have enough plants for the whole experiment. For the fertilizers, we have used the Flora Series fertilizers from General Hydroponics Company and fertilizers from the Atami Company. Namely: FloraGro (for the vegetative period), FloraBloom (for the flowering period), FloraMicro (for faster absorption of the nutriens and for stabilization of PH and EC), Flora Kleen (for removing the rests of the fertilizers in the water or in the substrate) and Ripen which is a bloom booster that stimulates the ripening processes. For the Atami Company we have used Rootblastic (helps plants to become deeply rooted) and Bloombastic which supports the flowering period and supplies the flowers with sugars and nutrients.

Figure 12: Fertilizers of the General Hydroponics and Atami company

4.2 Outdoor

For outdoor cultivation we have used three cuttings of *"The Doctor"*. According to Decorte (2010) the outdoor yield is usually expressed in g per plant so it is not necessary to have big outdoor plantations if you want to determine the outdoor yield in your climate conditions. The plants were planted at the beginning of May (3rd) and harvested at the end of September, earlier than the greenhouse plants because of lower temperatures outside and higher air humidity which were dangerous for the plants because of the risk of the moulds. The dosage of the fertilizers for outdoor cultivation is shown at the picture where the only difference is that the vegetative period lasted for three month not three weeks. After the harvest the plants were put into a dark place upside down for two weeks to become sufficiently dry.

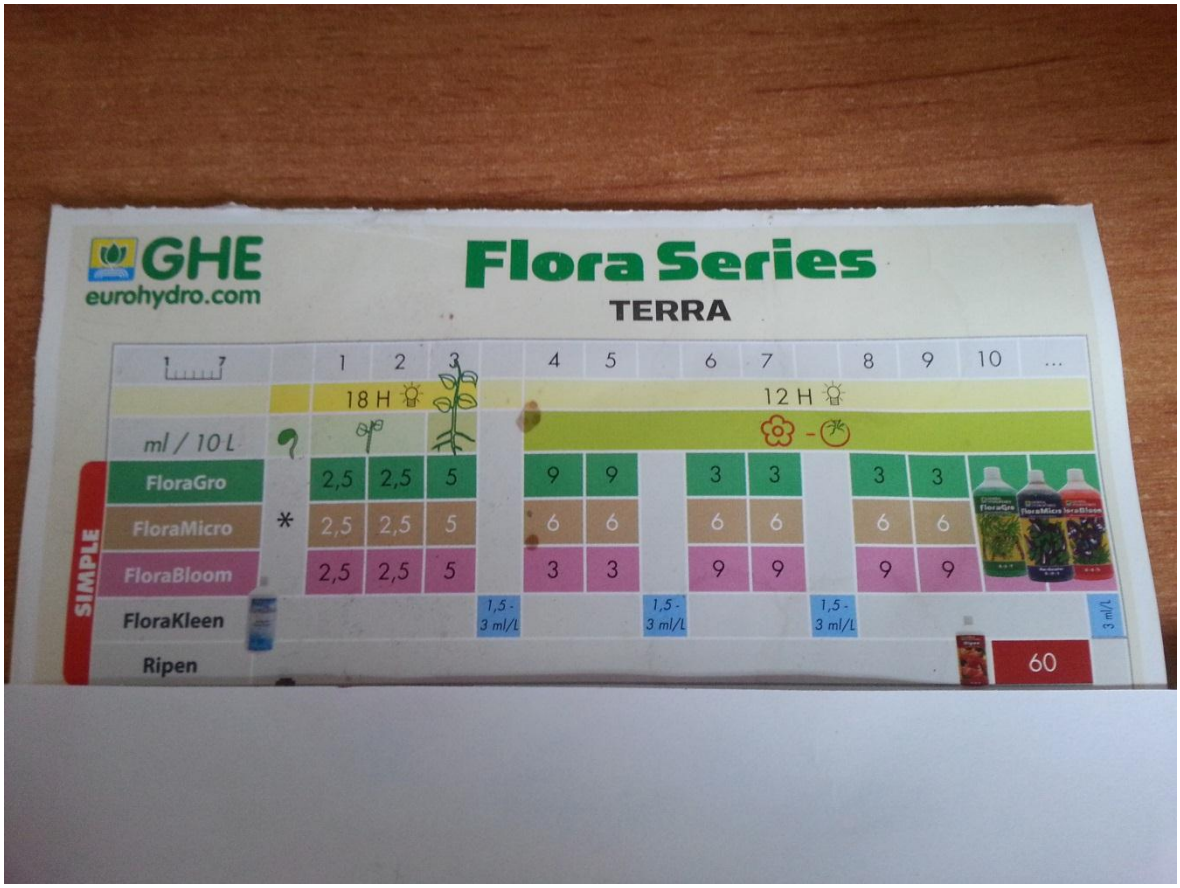

Figure 13: Table of fertilizer dosage used in our outdoor growth

4.3 Outdoor (greenhouse)

We put two cuttings of *"The Doctor"* to the greenhouse on the same day as for the outdoor cultivation (May 3rd) and we have also used the same dosage of fertilizers according to the schedule above. The main difference was that the greenhouse plants had a glass protection which was very useful in the late flowering period because it had reduced the risk of frost or heavy rains and therefore devaluation of the flowers. Therefore the greenhouse plants were harvested on the 17th of the October and had become dry the same way as the outdoor plants.

4.4 Indoor

For the first indoor cultivation we have used 30 cuttings of *"The Doctor"* which were placed in hydroponic system (Dutch pot system 1m²). Other necessary things for were the light (Philips SON-T PIA Plus 600W), light shield (Waweflector XL), Electrical ballast (ETI UAL 600 W), ventilator (TT 125 U 280m3/hour), fan (TRISTAR VE-5948) and of course the growing tent (Dark Street II 120x120cm). The average temperature and humidity was recorded with the thermometer (Eurotech IT-202). The plants were planted on the same day as the outdoor and greenhouse ones (May 3rd). Indoor plants were in the vegetative period (18 hours of light, 6 hours of dark) just one day before we turned the light on the flowering period (12-12hours). Therefore the harvest was very soon (6th of July). The dosage of fertilizers was therefore different than in outdoor cultivation (according to the picture below).

Figure 14: Table of fertilizer dosage used in indoor growth

4.5 Hemp ointment

The hemp ointment was made according to the method which was described in the literature review by Arjam Roskam and his colleagues from Green House Seed company. We used 10 grams of plant material (dry buds) on 200 grams of medical Vaseline in all four cases ("*The Doctor*" outdoor, "*The Doctor*" indoor, "*The Doctor*" greenhouse and The *„The Great White Shark"* indoor).

The plant material was coarsely crushed (not finely otherwise a lot of plant material will remain in the ointment). Then we made the water bath and put the Vaseline inside and waited till it has melted. Then we have added the plant material. We boiled the mixture approximately for two hours and then we have left it to cool down in dark, dry place with room temperature. After five days we repeated the whole procedure. We add Small amount of plant material with every additional repeating of the procedure. We repeated the whole process 4 times. After the last procedure we filtered the mixture through linen cloth to the bowls and have left it to become stiffer. We have done this process the same way in all four cases of plant material. The average weight of the finished ointment was 185.5 grams. That is because some ointment have been left in the plant material and

could not been properly squeezed out of the linen cloth. We made 742 grams of the ointment in total.

4.6 GC analysis

For the determination of cannabinoids (THC and CBD) the method described by UNODC (2009) was used. The laboratory analysis was done in cooperation with Department of Crop Production, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague.

Two hundred mg of dry and homogenized herbal hemp are extracted with 20 ml internal standard (ISTD) solution for 15 minutes in an ultrasonic bath. Due to the higher THC concentration in hemp resin, only 100 mg resin is needed. If the sample is liquid hemp (hemp oil), a weight of about 50 mg is sufficient. Decarboxylation of THC was done prior to the GC analysis. 500 µl of the solution is transferred to a 2ml GC vial. The vial is put into a heating unit (150 °C) for 12 minutes where the solvent is evaporated and the THC is decarboxylated. The residue is dissolved in 1.5 ml ethanol, the vial is shaken well and the resulting solution is then analysed by GC.

The determination of THC and CBD was done by GC- MS using Finnigan Focus GC (Thermo Electron Corporation, Madison, WI, USA.) with a DB-5MS column (15 m × 0.25 mm, 0.25 µm film thickness). The operating conditions were: injector temp. 280°C; carrier gas H (1 ml/min) constant flow; oven temperature: 2 min at 200°C, 10°C/min 200-240°C, 2 min at 240°C. Detector: FID 300°C, H_2 35 ml/min, Air 350 ml/min, internal standard: Tribenzylamine (TBA) in ethanol (0.5mg/ml), purchased from Sigma Aldrich (CZ), injection: 1.5 µl, split. Standards: CBD and THC purchased from Sigma Aldrich (CZ) were diluted in ethanol.

5 RESULTS

The whole experiment consisted of 60 hemp plants cultivated indoor (30 plants of *"The Doctor"* and 30 plants of *„The Great White Shark"*) and 5 hemp plants cultivated outdoor (3 plants of *"The Doctor"* in outside conditions and 2 plants of the same cultivar in greenhouse conditions). The total weight of all dry plant material (dry buds) was 3202 grams. The cost of all material and electric energy together was 37 500 CZK in total. The concentration of CBD was not detected in any of our plants.

5.1 Outdoor

The outdoor experiment lasted 147 days (from the 3rd May to 27th September) and the plants were harvested a little bit earlier due to the low temperatures, high humidity and rains which could devaluate the harvest (fungal diseases). Highest outdoor temperature was recorded on the 8th of August (33 °C) an on the other hand the lowest temperature was recorded on the 26th of September (-1 °C during the night). The amount of water which was used for watering one outdoor plant was 115 l and the dosage of fertilizers used for this one outdoor plant was 55 ml of Flora Grow, 75 ml of Flora Micro, 105 ml of Flora Bloom, 230 ml of Flora Kleen, 50 ml of Ripen, 3ml of Rootblastic and 22 ml of Bloombastic. This dosage was exactly the same in all three outdoor plants and therefore the whole dosage for all outdoor plants (3) was 345 l of water with three times higher number of each fertilizer. The total weigh of the plant material (in our case dry buds) was 1114 grams. First plant had 412 grams, second 332 and third 370. The outdoor average was 371 grams per plant. The average THC concentration in the outdoor Doctor was 6.74 %. The total costs of the outdoor growth were 1700 CZK (Just fertilizers).

5.2 Greenhouse

The greenhouse experiment lasted 167 days (from the 3rd May to 17th October) and the plants (2) were harvested a little bit later than outdoor ones but even though the harvest

was a little bit earlier in general due to the high humidity in the late flowering period (70%) and therefore high risk of fungal diseases. Temperatures in the greenhouse were often higher than in outdoor grow but never went over 37 °C during the day and never went over 8 °C during the night. The amount of water which was used for watering one greenhouse plant was 143 l and the dosage of fertilizers used for this one greenhouse plant was 70 ml of Flora Grow, 85 ml of Flora Micro, 120 ml of Flora Bloom, 300 ml of Flora Kleen, 60 ml of Ripen, 3 ml of Rootblastic and 30 ml of Bloombastic. This dosage was exactly the same in both greenhouse plants and therefore the whole dosage for all greenhouse plants was 294 l of water with two times higher number of each fertilizer. The total weight of the plant material was 928 grams. First plant had 470 and second 458 grams. The greenhouse average was 464 grams per plant. The average THC concentration in the greenhouse Doctor was 14.49 %. The total costs of the greenhouse growth were again just 1700 CZK for the fertilizers.

5.3 Indoor

Indoor experiment (*"The Doctor"*) lasted 62 days (from the 3rd May to 4th July 2014) which was faster than we thought. The lowest measured temperature was 19 °C during the night period and the highest temperature (25 °C) was recorded during the light period. It should be mentioned that those temperatures were recorded regularly every day so there were no fluctuations or dramatic changes in temperatures during the whole growth. The amount of water which was used in the aeroponic system was 430 l and the dosage of fertilizers used for this indoor growth was 180 ml of Flora Grow, 200 ml of Flora Micro, 350 ml of Flora Bloom, 500 ml of Flora Kleen, 450 ml of Ripen, 100 ml of Bloombastic and 4 ml of Rootblastic. This amount was used for all 30 indoor plants together. It should be mentioned that some water could have evaporated due to the higher temperatures and direct light. The total weight of the dry buds was 579 grams. In our case the indoor average for *"The Doctor"* was 19.3 grams per plant. The average THC concentration for the indoor Doctor was 13.20 %. The total cost of the material needed for the first indoor growth (including the system with pots, fertilizers, electric energy ect.)

was 30 400 CZK where the cost of the fertilizers was 1700 CZK and the costs of the energy were 1500 CZK per month.

Second indoor experiment (*Great White Shark*) lasted 60 days (from 19th of July to 17th of September) which was even faster than in the previous indoor growth. The range of temperatures was exactly the same like in the previous growth (19-25 °C) and stable. The amount of water which was used for irrigation was 415 l and the dosage of fertilizers was 170 ml of Flora Grow, 185 ml of Flora Micro, 340 ml of Flora Bloom, 480 ml of Flora Kleen, 450 ml of Ripen, 90 ml of Bloombastic and 4 ml of Rootblastic. The amount was used together the same way as in the previous indoor growth for all 30 plants. The total weight of the dry buds was 581 in the case of *„The Great White Shark"*. The average was then 19.36 grams per plant. The average THC concentration for the indoor *„The Great White Shark"* was 14.65 %. The total cost of the material and electric energy was 4700 CZK (without the acquisition price of the system and other components which were already bought for the first indoor growth).

Table 1. Comparison of the different growing techniques: yield, quality of their products and operational costs

Type of cultivation ("The Doctor")	Yield (grams)	Length of cultivation (days)	THC content (%)	Costs of cultivation (CZK)
Outdoor	371/plant (1114 in total)	147	6.74	1700
Greenhouse	464/plant (928 in total)	167	14.49	1700
Indoor	579/m² (19.3/plant)	62	13.20	30 400

6 DISCUSSION

Vanhove and his team (2011) have posted several hypotheses and articles to explain and describe the main factors which determine the yield and quality of illicit hemp production. We found out that many of these factors have been also involved in our experiment. These are: amount of sunlight, amount of water and nutrients and finally the variety. However, Vanhove´s results confirmed quite big differences in the THC concentration in individual plants cultivated in the same conditions.

On the contrary our hypothesis H2: Content of cannabinoids will be more or less the same in both indoor cultivations (*"The Doctor"* and *„The Great White Shark"*) because the growing conditions were exactly the same – has been proved in our indoor conditions. That may be a result of more stable growing conditions than in Vanhove et al. (2011). On the contrary the hypothesis H1: The content of cannabinoids will be higher in indoor cultivation because the growing conditions (temperature, light, humidity etc.) is more stable in indoor than in outdoor growth in the Czech Republic has not been proved. It was a surprise for us, because we all had been expecting the exact opposite according to Toonen et al. (2006), Vanhove et al. (2011), Vanhove et al. (2012), even if Decorte (2010) and Consetino et al. (2012) thought that the temperature factor does not have that big role in THC content. Nevertheless, we have proved that the greenhouse conditions are more suitable than indoor for variety *"The Doctor*" (speaking about THC content) which means much lower investment for even a better product.

According to Consetino with colleagues (2012) and Decorte (2010) the indoor marihuana yield is expressed in number of grams per 1 square meter and the outdoor yield is expressed as number of grams per plant. Our average indoor yield was 580 grams/m² (579 - *"The Doctor"*, 581 – *„The Great White Shark"*). Moreover, according to Toonen et al. (2006) the average indoor yield is determined between 366 – 610 g/m² which means that both of our indoor experiments had been cultivated well (in case of yield).

Our other success was the comparison of the indoor yields in our experiment with other indoor yields from different experiments. Toonen et al. (2006) had average yield of indoor plantation 456 g/m² which is more than one hundred grams less than in our both indoor cultivations.

Vanhove and colleagues (2011) have achieved average 483.33 g/m² of dry indoor grown buds which is also about one hundred grams less than in our case. On the contrary in Vanhove et al. (2011) in the indoor experiment, the maximum THC concentration in the plant material (16.3% THC in the Super Skunk variety) was higher than in our experimental plant material (our highest THC concentration was found in *„The Great White Shark"* – 14,65 %). The concentration of CBD was not recorded in our studied plants as well as in Vanhove et al. (2011) - they achieved only a small concentration of CBD or none.

Hilling et al. (2004) also did not detect CBD concentration in most of their plant material but their GC analyses (in which more highly concentrated extracts were analysed) show that CBD was in fact present in all plants, but below the threshold of detection. This may also be the case in other studies that reported the absence of CBD in certain drug strains (Baker et al*.,* 1980; Baker et al., 1982).

7 CONCLUSION

Cultivation and subsequent production of the medicinal hemp products is still not yet fully developed and understood because there are many potential obstacles with the law and cultivation itself. Moreover there are problems with successful and stable production of the appropriate plant material (stable yield and THC content) for the medicinal purposes and the plant material is nowadays imported from to the Czech Republic from Netherlands. The unstable outdoor yields are a result of changing of internal factors (Genetic variability) and external factors (sunlight, temperature, humidity, wind, availability of water and nutrients, etc.). We do not need to solve the external factor problems in the case of indoor cultivation because it is easier to achieve stable indoor conditions without any fluctuation than in outdoor cultivation. However the indoor plants are being much more dependent on the person who takes care of them.

Among all of our cultivated plants the biggest success was achieved in the greenhouse Doctor cultivation where we harvested 928 grams with the average of 464 g/plant and the THC concentration (14.49%) was even higher than in the indoor cultivated Doctor (13.20%). And the biggest and the most important difference was in the operational costs where the greenhouse "*The Doctor*" cost just 1700 CZK (the cost of fertilizers) and the indoor Doctor operational costs were 30 400 CZK which is an astronomically higher price. On the contrary, if we take into consideration the length of the cultivation period, the indoor cultivation (60-62 days) had almost three times shorter cultivation period than the outdoor cultivation (147-167).

For successful cultivation of the *Hemp* for its medicinal uses in Czech Republic I would definitely recommend the greenhouse cultivation because of a lower price and even better product (in case of *"The Doctor"*). The greenhouse temperatures should not drop below 8 °C; quite fertile soil (unless if you apply adequate dosage of fertilizers), enough space, appropriate cultivar and sunlight are the key factors of determining the yield and yield quality.

In case of further laboratory research I would highly recommend the hydroponic indoor cultivation even though the initial costs are high, because it is much faster and that comes in handy when it is necessary to cultivate more cultivars in the same conditions in one year or less. The other reason is that the fertilizers are added equally to all plants together and the service of the system is hardly needed.

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