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Assessment of cycocel effects on biochemical characteristics of characteristics medical plant, *Cannabis sativa* L in flowering stage

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Abstract

Cannabis Sativa Lis one of the oldest farm plants that have both medicine and industrial uses.in this study we investigated the In this study, the cycocel solution (0, 500, 100, and 1500 mg/l)was sprayed on the plants in 2 periods within 10 days of each other, during the 5 pair-leaves stage. We measured theDelta-9-tetrahydrocannabinol, soluble carbohydrates and proteins content. Results showed that with increase the regulator level of cycocel to 500 mg/l, tetrahydrocannabinol (THC) in active ingredient of cannabis leave and flower in female plant increased significantly. From the comparison of interaction between genders and cycocel can be concluded that the effective material of leave in female gender shown the better results toward the male gender until 1000 mg/l concentration. The control treatment had the minimum of effective material value in female gender that shown significant difference with 500 mg/l treatment. The cycocel densities in comparison to the control increased the soluble carbohydrates, which were significant for all treatments comparing to the control. The female plants had higher amount of soluble carbohydrates in comparison to the male ones. Results indicate that the 1000 mg/l treatment had the highest protein amount in comparison to both the male and female plants in other treatments. Until the 1000 mg/l, the male was higher than the female, but at 1500 mg/l, the female plant fell ahead.

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Introduction

The cannabis (*Cannabis Sativa L.*), is a dual-base, single year plant from the Urticales order, the Cannabaceae family. This plant has claw-shaped leaves, with 5-7 serrated leaflets (Yoshi Matsu and Kitazawa, 2004). Using the plant growth regulators causes the gender to change in elder cannabis plants. Such that the Gibberellin causes the plant to be male, and the Abscisic acid stimulates the female plants, or bisexual flowers (Chailakhyan and Khryanni 1978).

The use *Cannabis sativa* (cannabis) extracts as medicine was described in China and India (Mikuriya., 1969) before the birth of Christ. The therapeutic use of cannabis was introduced in Western medicine in the first half of the 19th century and reached its climax in the last two decades of the same century. At the turn of the century, several pharmaceutical companies were marketing cannabis extracts and tinctures which were prescribed by doctors for many different complaints including pain, whooping cough and asthma, and as a sedative/hypnotic agent (Fankhauser., 2002). However, the use of cannabis as a medicine almost completely disappeared at about the middle of the 20th century. The main reasons for this disappearance were the variable potency of cannabis extracts, the erratic and unpredictable individual responses, the introduction of synthetic and more stable pharmaceutical substitutes such as aspirin, chloral hydrate and barbiturates, the recognition of important adverse effects such as anxiety and cognitive impairment, and the legal restrictions to the use of cannabis-derived medicines (Fankhauser., 2002).

Cannabis has many medical uses. Cannabinoids are terpenophenolic compounds that have been identified only atthe cannabisgenus (Croxford and Yamamura, 2005). Over than 60cannabinoids has been identified at the cannabis. From the main cannabinoidscan name of Cannabigerol (CBG), Delta-9-tetrahydrocannabinol (THC), Canabinol (CBN), Cannabidiol (CBD) and Cannabichromene (CBC) (Yoshimatsu and Kitazawa, 2004). Among these components, most of the medical effect is related to THC (Croxford and Yamamura, 2005).

Today this situation has changed considerably. The main active psychotropic constituent of cannabis, D^9-tetrahydrocannabinol (D^9-THC), was isolated, identified and synthesized in the 1960's. Almost three decades later, cannabinoid receptors in the brain were described and cloned and the endogenous cannabinoids were isolated and identified (Martin *et al.,* 1999).

Although D^9-THC is commonly accepted as the main factor responsible for the effects of cannabis, several reports have demonstrated that other components of the plant influence its pharmacological activity (Carlini *et al.,* 1970). One of these components is cannabidiol (CBD), which may constitute up to 40% of cannabis extracts (Grlie., 1976) and is devoid of the typical psychological effects of cannabis in humans (Zuardi *et al.,* 1982). Studies on the interaction between D^9-THC and CBD have produced apparently contradictory results (Karniol & Carlini, 1973). Although potentiation of the effects of D^9-THC has been observed (Fernandes *et al.*, 1974; Hollister & Gillespie., 1975), this phenomenon probably involves pharmacokinetic interactions since CBD is a potent inhibitor of hepatic drug metabolism (Bornhein *et al.*, 1981) and increases D^9-THC concentrations in the brain (Jones & Pertwee, 1972). This research was aimed to investigate the changes Cycocel Effects on D^9-THC, soluble carbohydrates and protein of Medical Plant, *Cannabis Sativa* L .in Flowering Stage.

Methods and materials

This study was inducted with the goal of analyzing the effect of different cycocel densities, on the cannabis medical plants stereotypes. In this test, we used pots with the diameter of 20 cm, and the height of 35 cm. To prepare the planting bed, a mixture was made including 33% perlite, 33% humus, and 33% regular garden soil, which was mixed into a uniform state. In each pot, 6 seeds were planted, and after two weeks, the count of plants within each pot decreased to 1. The cycocel solution was sprayed on the plants in 2

periods within 10 days of each other, during the 5 pair-leaves stage (the plant and the soil beneath it were soaked in the solution). The plants were permitted to grow till their flowering stage. After blooming, 2/3 of the male blossoms from the male plants, and all the female blossoms from the female plants were harvested. This study is inducted in the block factorial test formation, in the format of completely random blocks, at 4 cycocel hormone levels (0, 500, 1000, and 1500 mg/l), on both genders, with 3 replications for each plant separately.

Extraction THC50

For extraction, THC50 mg of leaf of flower dry texture with 1ml chloroform was centrifuged for 15 minutes. The solvent was evaporated after filtrate extract and the remains resolved in 5.0 ml methanol. The quantitative measurement of THC done with 1.7 micrometer diameter of particles by Liquid Chromatography method and Ultra-Performance Liquid Chromatography (UPLC) set made in Waters Company with PDA detector and UPLC BEH C_{18} (2.1 mm × 150 mm) column. For analysis of THC used of acetonitrile slope of water (PH=305.0) with TFA percent buffer from 70:30 until 100:0 in 5 minutes, 100:0 in one minute, back to 30:70 in one minute with 4.0 ml min^{-1} flux, 230nm wavelength and µl7injection volume.

measure protein amount

To measure the protein amount, we used the Bradford method (1976). For this means, we added 0.1 ml protein essence and 5 ml of the Biuret reagent to a test tube, and immediately subjected it to vortex. After 2 minutes, and before one hour, its absorption was read via a spectrometer device, set at 595 nm, and the protein density was measured using the standard curve, and calculated based on mg/g (wet weight).

measure the soluble carbohydrates

To measure the soluble carbohydrates, we used the Fales method (1951). We extracted the soluble carbohydrates from 0.1 g of the shoot, alongside 2.5 ml 80% ethanol, which were heated at 90^{O}C, for 60 minutes (two 30 minutes stages), and the essence was cleared using filter paper, an afterwards the alcohol was vaporized. The resulted sediment was dissolved in 2.5 ml of pure water. From each sample, 200 µl were poured in test tubes, and after wards mixed with 5 ml of the Anthrone reagent. After mixing, they were placed in 90^{O}C for 17 minutes, and after cooling off, their absorption were read at 625 nm. The density of each sample was calculated via the standard curve, according to mg/g (wet weight).

Statistical analysis

The variance analysis of the data was done via the SPSS *ver 16* software, and the averages were compared via the Duncan test on the (P>0.05) level.

Results and discussion

Delta-9-tetrahydrocannabinol (THC)

Active ingredient of cannabis leaf

Tetrahydrocannabinol (THC) is the most important of active ingredient in cannabis that use in treatment of diseases. With increase the regulator level of cycocel to 500 mg/lit, leaf tetrahydrocannabinol (THC) of cannabis active ingredient in female genus was increased significantly (Diagram1). According to tetrahydrocannabinol (THC) index, the statistical difference was between the male and female genus at all inhibitor levels of cycocel as get to its maximum value in 500 mg/lit concentration in female genus and this value in male genus was in 1500 mg/l concentration.

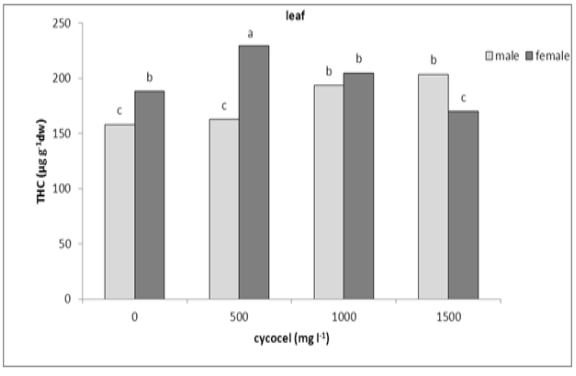

Fig. 1. The cycocel effects on tetrahydrocannabinol (THC) in leaf *C. Sativa*.

With comparing the interaction between genus and cycocel can conclude that the female genus has been

has shown the better results toward male genus until 1000 mg/lit concentration. The control treatment had the minimum value of active ingredient in female genus that shown significant difference with 500 mg/lit treatment.

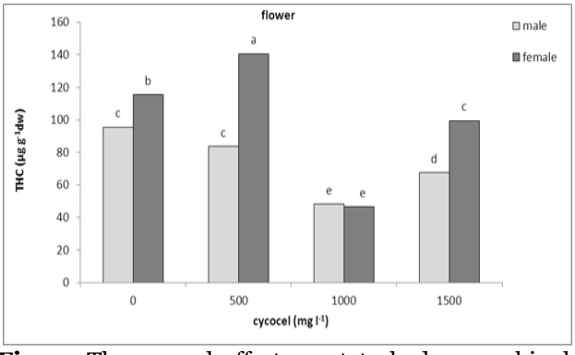

Fig. 2. The cycocel effects on tetrahydrocannabinol (THC) in flower *C. Sativa*.

Active ingredient of cannabis flower

With increase the regulator level of cycocel to 500 mg/l, the active ingredient of cannabis was increased significantly. According to tetrahydrocannabinol (THC) index, the statistical difference was between the male and female genus at all inhibitor levels of cycocel as get to its maximum value in 500 mg/l concentration. With comparing the interaction between genus and cycocel can conclude that the female genus has been has shown the better results toward male genus. With increase the cycocel concentration from 500 mg/lto 1000 mg/lit, the active ingredient of cannabis decreased significantly. Similar with the obtained resultsin this experiment, Hackoy *et al* (2007) reported that the cycocel caused the increase of *pyrethrin value* (monoterpene) in *chrysanthemum cinerariaefolium*.

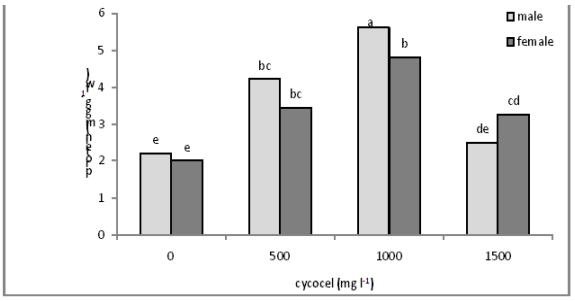

Fig. 3. The cycocel effects on protein content in *C. Sativa*.

Despite the importance of cannabis as a medical plant yet has not conducted the scientific research on active ingredient of native cannabis in Iran. In this study THC value examined.

This study is the first report about investigate the native cannabis of Iran to THC value and plant hormones on change the value of this blend in plant. The results showed according to the ration between THC values by Karl *et al* (2004) was defined as a criterion for determination of medical and fibrous types of this plant, the native cannabis of Iran with THC/CBD ratio over one is in medical group. THC usually is found at young leaves with low amount and this blend mostly is in the form of acidic compound of tetrahydrocannabinol acid (THCA) that convert to THC by non-enzymatic decarboxylation during of drying or store of plant tissues (Sirikantramas *et al.*, 2004).

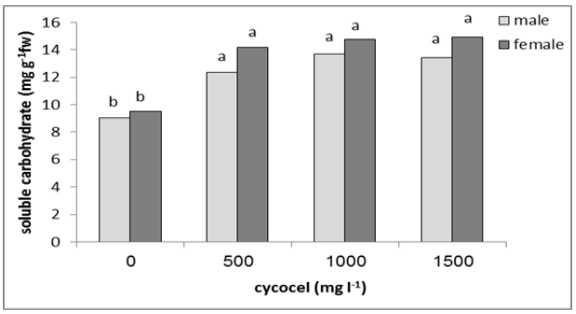

Fig. 4. The cycocel effects on soluble carbohydrates in *C. Sativa*.

Protein content

Results indicate that the 1000 mg/lit treatment had the maximum amount of protein in comparison to other treatments, either the male or female plants. While comparing the male and female plants, the male was higher up to the 1000 mg/lit, but at 1500 mg/lit, the female plant fell ahead. The 1000 mg/lit treatment indicated a significant difference with the observer treatment. Kumar and colleagues (1990) reported that the accumulated amount of protein in the sunflower plant, increase by 80% when applying cycocel.

Carbohydrates content

The cycocel densities increased the amount of soluble carbohydrates in comparison to the observer treatment, which all treatments became significant comparing to the observer one. The female plants had

a higher amount of soluble carbohydrates comparing to the male plants.

Kumar and colleagues (1990) showed that the accumulated amount of soluble Carbohydrates in the sunflower plant increases by 80%, while using cycocel. It could be said that the most important factor to increase the TSS amount, caused by the use of cycocel during the drought stress, is the destruction of insoluble carbohydrates by the Abscisic acid, which are synthesized by the cycocel, and eventually lead to an increased amount of TSS.

References

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantites of protein utilizing the principle of protein-dye binding. Analytical Biochemistry **72,** 248-254.

Bornhein LM, Borys HK, Karler R. 1981. Effect of cannabidiol on cytochrome P-450 and hexobarbital sleep time. Biochemical Pharmacology **30,** 503-507.

Carlini EA, Santos M, Claussen V. 1970. Structure activity relationship of four tetrahydrocannabinols and the pharmacological activity of five semipurified extracts of *Cannabis sativa*. Psychopharmacologia **18,** 82-93.

Chailakhyan MKH, Khryanni VN. 1978. Influence of growth regulators absorbed by the root on sex expression in hemp plants. Planta **2,** 181-184.

Croxford JL, Yamamura T. 2005. Cannabinoids and the immune system: Potential for the treatment of inflammatory diseases? Journal of Neuroimmunology **166,** 3-18.

Davis WM, Borgen LA. 1974. Effects of cannabidiol and □9-tetrahydrocannabinol on operant behavior. Research Communications in Chemical Pathology and Pharmacology **9,** 453-462.

Fales, FW. 1951. The assimilation and degradation of carbohydrates by yeast cells. Journal of Biological Chemistry **193,** 113-124.

Fankhauser M. 2002. History of cannabis in Western Medicine. In: Grotenhermen F & Russo E (Editors), *Cannabis and Cannabinoids*. The Haworth Integrative Healing Press, New York, 37-51.

Fernandes M, Schabarek A, Coper H. 1974. Modification of □9-THC-actions by cannabinol and cannabidiol in the rats. Psychopharmacologia **38,** 329-338.

Grlie L. 1976. A comparative study on some chemical and biological characteristics of various samples of cannabis resin. Bulletin on Narcotics **14,** 37-46.

Hollister LE, Gillespie H. 1975. Interactions in man of □9-tetrahydrocannabinol, H-cannabinol and cannabidiol. Clinical Pharmacology and Therapeutics **18,** 80-83.

Jones G, Pertwee RG. 1972. A metabolic interaction *in vivo* between cannabidiol and □9-tetrahydrocannabinol. British Journal of Pharmacology, **45,** 375-377.

Karniol IG, Carlini EA. 1973. Pharmacological interaction between cannabidiol and □9-tetrahydrocannabinol. Psychopharmacologia **33,** 53-70.

Karniol IG, Shirakawa I, Kasinsky N. 1974. Cannabidiol interferes with the effect of □9-tetrahydrocannabinol in man. European Journal of Pharmacology **28,** 172-177.

Karl W, Mahlberg H, Mahlberg PG. 2004. A chemotaxonomic analysis of cannabinoid variation in cannabis (Cannabiacea). American Journal of Botany **91(6),** 966–975.

Kumar J, Pal K. 1990. Effect of Cycocel on growth,yield and quality of Tomato

(Lycopersiconesculentum) .Departments Charan Singh University Campus.Meerut India .

Martin BR, Mechoulam R, Razdan RK. 1999. Discovery and characterization of endogenous cannabinoids. *Life Sciences* **65,** 573-595. http://dx.doi.org/10.1016/S0024-3205(99)00281-7

Mikuriya TH. 1969. Marijuana in medicine: past, present and future. California Medicine **110,** 34-40.

Sirikantaramas S, Morimoto S, Shoyama Y, Ishikawa Y, Yoshiko Y, Shoyama Y. 2004. The gene controlling marijuana psychoactivity molecular cloning and heterologous expression of tetrahydrocannabinolic acid synthase frome cannabis sativa L. Journal of Biological Chemistry. 39767-39774. http://dx.doi.org/10.1107/S1744309105023365

YoshimatsuK Iicla O, Kitazawa T. 2004. Growth characteristics of Cannabis sativa cultivated in a phytotron and in the field. Bulletin on Natural Instruction of Health Science **122,** 16-20.

Zuardi AW, Shirakawa I, Finkelfarb E. 1982. Action of cannabidiol on the anxiety and other effects produced by □9-THC in normal subjects. Psychopharmacology **76,** 245-250.

Zuardi AW, Finkelfarb E, Bueno OFA, 1981. Characteristics of the stimulus produced by the mixture of cannabidiol with D9-tetrahydrocannabinol. Archives Internationales de Pharmacodynamie et de Therapie **249,** 137-146. http://dx.doi.org/10.1590/S0100879X2006000400001