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Effect of cycocel on growth retardant cycocel on reducing sugar, malondialdehyde and other aldehydes of *Cannabis Sativa* L.

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Abstract

Cannabis Sativa L. is one of the oldest farm plants that have both medicine and industrial uses.in this study we investigated the In this study, the cycocel (CCC) solution (0, 500, 100, and 1500 mg L^{-1}) was sprayed on the plants in two periods within 10 days of each other, during the 5 pair-leaves stage. We measured the reducing sugar, malondialdehyde and other Aldehydes content. Results showed that with increase the regulator level of CCC to 1000 mg L^{-1}, reducing sugar in active ingredient of cannabis leave in male plant increased significantly. From the comparison of interaction between genders and CCC can be concluded that the reducing sugar of leave in male gender shown the better results toward the female gender until 1000 mg L^{-1} concentration. The control treatment had the minimum of malondialdehyde and other Aldehydes content value in female gender that shown significant difference with 1000 mg L^{-1} treatment. The male plants had higher amount of malondialdehyde in comparison to the female ones.

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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).

Cycocel (2-Chloroethyl, trimethyl ammonium chloride) has been used to check the abscission of flower and modify the crop canopy for improving the yield in gram (Bangal *et al*., 1982), pigeonpea (Vikhi *et al*., 1983) and soybean (Singh *et al*., 1987). Grewal*et al*., (1993) reported that cycocel improves the translocation of photosynthates. More protein content stored in the seeds might be due to improvement of translocation of photosynthates to the seeds.

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 (salehi sardoei *et al*., 2014).

El-Sabrout (1996) reported that foliar application of cycocel (500, 1000 and 1500 ppm) increased reducing, non-reducing and total sugar content of leaves while starch and total carbohydrates content decreased with increasing concentration of growth retardants. Similarly, the application of cycocel (0 – 0.8%) increased total soluble sugars, protein and starch content in sunflower leaves (Kumari *et al*., 1990). Szynal *et al* (2001) reported that the application of CCC increased reducing sugar content in wheat seedling.

This research was aimed to investigate the changes CCC Effects on Reducing Sugar, Malondialdehyde and Other Aldehydes of Medical Plant, *Cannabis Sativa* L.

Materials and methods

Cultivation Conditions

This study was inducted with the goal of analyzing the effect of different CCC 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.

Treatments

The CCC 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 CCC hormone levels (0, 500, 1000, and 1500 mg L^{-1}), on both genders, with 3 replications for each plant separately.

Reducing sugars

Glucose and fructose containing aldehyde and ketone groups can be oxidized by some materials. Sugars containing free anomeric carbons are called reducing sugars. In this experiment, presence of reducing sugars reduced Cu^{+2} to Cu_2O. Cu_2O reduces phosphomolybdic acid which produces blue color formation. Severity of produced color which is positively correlated with reducing sugars concentration can be evaluated by spectrophotometer. Somogy method (1952) was used to determine the concentration of reducing sugars. 0.02 g of aerial part was pulverized with 10ml of distillated water. The mixture was transferred in to a small beaker and heated on electrical stove. Heating was stopped when the mixture reached boiling point; content of the beaker was filtrated by whatman filter paper no.1 to obtain plant extract. 2 ml of the plant extracts was transferred to test tube, 2 ml of copper sulphate was added, the tubes were sealed with cotton and incubated for 20 min in water bath 100$^{\circ c}$. in this step, Cu^{+2} is transformed in to Cu_2O by reduced aldehyde monosaccharide and a brick red color is observed. When the tubes were cooled, 2 ml of phosphomolybdic acid was added and blue color appeared. The test tubes were thoroughly agitated until the color was evenly distributed in the tube. Absorbance was determined in 600 nm by spectrophotometer and concentration of the reducing sugars was calculated by drawing standard curve. The results were calculated and reported as mg per g of fresh weight.

Drawing standard curve

To draw standard curve, concentrations of 5, 10, 20, 40, 60 and 100 mg L^{-1} of glucose were prepared and 2 ml of each concentration was poured in clean test tube. Other steps were performed as for unidentified samples and solution absorbance was read by spectrophotometer in 600 nm. Absorbance curve was drawn against concentration and the line equation was achieved.

Preparation of copper sulphate solution

40 g of anhydrous sodium carbonate was dissolved in 400 ml of distillated water and added to 7.5 g of tartaric acid. After dissolving in acid, 4.5g of $CuSO_4.5H_2O$ was added and final volume was increased to 1 liter.

Preparation of phosphomolybdic acid solution

70 g of phosphomolybdic acid and 10 g of sodium tungstate were dissolved in 700 ml of 5% hydroxide sodium and heated for 40 min. when the solution was cooled, 250 ml of 85% phosphoric acid was added and the final volume was increased to1 liter.

Malondialdehyde concentration

Malondialdehyde (MDA) concentration was measured by method Heath and Packer (1969). 0.2 g of frozen plant tissue (stem and leaf) was pulverized in 5 ml of 1% trichloroacetic acid (TCA). The solution was centrifuged for 5 min in 10000 g. 4 ml of 20% TCA containing thiobarbituric acid (TBA) was added to the supernatant. The solution was incubated for 30 min in water bath 95$^{\circ c}$ and immediately cooled in ice. The mixture was then centrifuged in 10000 g for 10 min. absorbance of solution was evaluated in 532 nm using spectrophotometer Varian Cary 50 device (Germany). The target substance for absorbance in this wavelength is MDA-TBA red complex. Absorbance of other non-specific pigments was determined in 600nm and deducted from this value. Extinction coefficient equal to 155 $mM^{-1}cm^{-1}$ was used to determine MDA concentration, and the results were calculated as nanomole per gram of fresh weight.

Concentration of other aldehydes (propanal, butanal, hexanal, heptanal and propanal dimethyl acetal)

Concentration of other aldehydes was measured using method Meir *et al* (1992). 0.2 g of frozen tissue was pulverized in 5ml of trichloroacetic acid. The mixture was centrifuged in 10000 g for 5min and 4ml of 20% TCA containing 0.5% thiobarbituric acid was added to the supernatant. The solution was incubated for 30 min in water bath 95°c and immediately cooled in ice. The mixture was then centrifuged in 10000 g for 10 min. the cooled solution was centrifuged in 10000 g for 10 min and its absorbance was measured in 455 nm. Absorbance of other non-specific pigments was read in 600 nm and subtracted from the value. Extinction coefficient equal to 0.458×10^5 $mM^{-1}cm^{-1}$ was used to determine concentration of the aldehydes. Results were calculated as nanomole per gram of fresh weight.

Statistical analysis

Analysis was performed on data using SPSS ver 16. Comparisons were made using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Differences were considered to be significant at $P < 0.05$.

Results and discussion

Effect of gender

Regardless of CCC effects, it seems that there is no difference among the indices measured in cannabis genera except for reducing sugars. Soluble carbohydrates and Malondialdehyde had the most number of indices in female plant but there was no significant difference from male plants (table1).

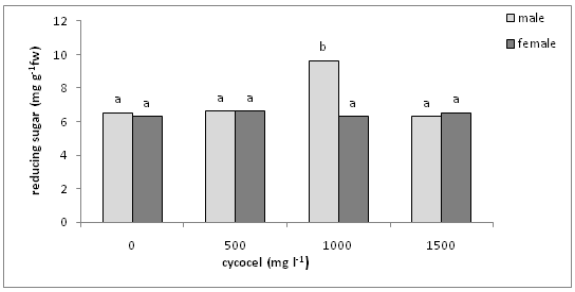

Fig. 1. The CCC effects on in Reducing Sugar *C. Sativa*.

Effect cycocel

Table (1) shows that all the indices were elevated by increase in CCC concentration and at the concentration of 1000 mg L^{-1}, protein and soluble sugar content were reduced. The high rate of soluble carbohydrates was achieved in 500 and 1500 mg L^{-1} of CCC. The highest content of other aldehydes was obtained in 500 mg L^{-1} showing significant difference from control (distillated water). According to results presented in table (1), all the chemical parameters were statistically affected and significant.

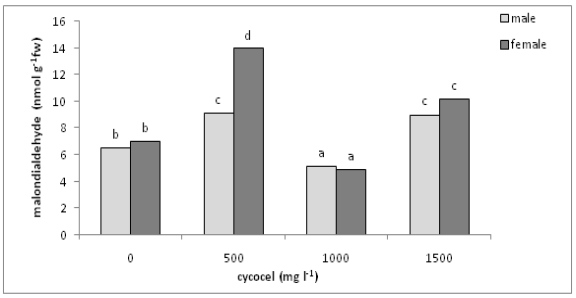

Fig. 2. The CCC effects on Malondialdehyde in *C. Sativa*.

Reducing Sugar

With a significant difference from other treatments, application of 1000 mg L^{-1} of CCC increased reducing sugars in male plants. In female plants, there was no significant difference between any treatments and control group (Diagram 1). El-Sabrout (1996) showed that application of CCC in concentrations of 500, 1000 and 1500 mg L^{-1} as foliar spray increased reducing and non-reducing sugars and total carbohydrate content of leaves; whereas starch and total carbohydrate content was reduced due to application of growth retardants. Szynal *et al* (2001) reported that foliar application of CCC increased reducing sugar content in wheat seedlings.

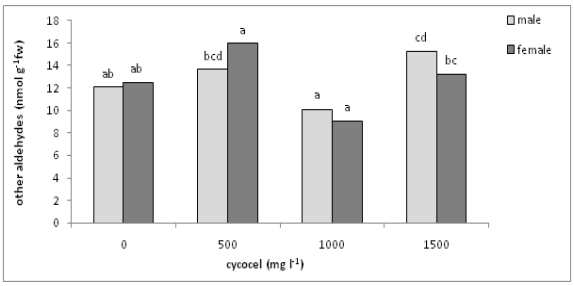

Fig. 3. The CCC effects on other Aldehydes in *C. Sativa*.

Abiotic stresses cause change in carbohydrate content whose amount is positively correlated with photosynthesis. As a physiologic process,

photosynthesis has the highest sensitivity to high temperature. The result of increased temperature and consequent damages is disequilibrium between photosynthesis and respiration. In general, increased temperature results in reduction of photosynthesis and increase in respiration photorespiration (Pancheva and Popova., 1998).

Under stress condition, plant respiration is increased and plant demands more substrate to produce energy. Moreover, heat stress has significant influence on biosynthesis of starch and sucrose by reducing activity of sucrose synthase, ADP-glucose pyrophosphorylase and invertase. Regarding reduced photosynthesis and declined content of soluble sugars, carbohydrate stores are concerted to soluble sugars. Since soluble carbohydrates are cellular osmolytes, increase in soluble sugar content is effective in water retention and prevention of dehydration (Camejo *et al.*, 2005). Accumulation of soluble sugars in geranium leaves increased accumulation of starch for retention of cell turgescence. When water potential in a leaf is reduced, accumulation of sugars probably plays the main role of osmotic adjustment (Arora *et al.*, 1998). In the present study, it looks that CCC at the concentration of 1000 mg L^{-1} can assist osmotic adjustment of male plant by increasing sugar content. Kumar and Pal (1990) showed that the accumulated amount of soluble Carbohydrates in the sunflower plant increases by 80%, while using CCC. It could be said that the most important factor to increase the TSS amount, caused by the use of CCC during the drought stress, is the destruction of insoluble carbohydrates by the ABA, which are synthesized by the CCC, and eventually lead to an increased amount of TSS.

Plant growth regulators (growth promoter and growth retardants) are known to regulate the metabolism in the plant by increasing the duration of the source there by maintaining the proper balance of source and sink. The degree of perfect physiological relations indirectly affect the flowering without causing malformation in the plants. In this connection, application of growth retardants to optimize plant production by modifying growth, development and the quantitative and qualitative yield of crop plant hold promise and sunflower is not an exception for this.

The increase in the sugar content with advancement in age could be due to stimulation of amylase and other hydrolytic enzymes promoting the hydrolysis of storage reserves due to senescence. It is expected that with advancement in the crop growth, metabolic activity of the plants is increased to support the reproductive growth.

Malondialdehyde (MDA) and other aldehydes (propanal, butanal, hexanal, heptanal and propanal dimethyl acetal)

These compounds are produced by oxidation of membrane lipids. Changes in Malondialdehyde and other aldehydes indicate damage to cell leaking. Malondialdehyde content was significantly increased by application of 500 and 1500 mg L^{-1} CCC. The lowest malondialdehyde content was achieved in both male and female plants by 1000 mg L^{-1} of CCC. The male and female plats showed significant difference in 500 mg L^{-1} but the difference was not significant in control. Concentration of 500 mg L^{-1} was significant in all the CCC concentrations (Diagram 2). Considering that gibberellins and phytosterols are biosynthesized in the same pathway, it is expected that inhibition of gibberellin biosynthesis pathway can influence phytostrol content and consequently membrane health. In this experiment, application of CCC increased malondialdehyde, propanal, butanal, hexanal, heptanal and propanal dimethyl acetal produced by lipid oxidation which indicates damages caused by active oxygen species such as superoxide radical, hydrogen peroxide and hydroxide radical to cell membrane. Production of active oxygen species causes lipid peroxidation and damage to cell membranes as the first mechanical barrier against environmental stresses. The highest content of other aldehydes was achieved by application of 500 mg L^{-1} which was higher in female plants than male counterparts (Diagram 3).

There are currently five recognized groups of plant growth regulators *viz.,* auxin, gibberellins, cytokinins, abscissic acid and ethylene. The natural phytohormones are involved in growth and differentiation, auxiliary bud growth, root elongation, shoot elongation, cell division, flower initiation *etc*. Besides to this there are several synthetic bio-regulators that regulate growth and development and behavior of plant without inducing phytotoxic or malformative effects. These bio-regulators comprises of both retardants and promoters which when used in appropriate concentrations, influence the plant architecture in a typical fashion. Dicks (1980) characterized the phenomenons which are influenced by growth retardants such as inhibition of shoot growth (plant height, internode elongation and leaf area) with unchanged number of internodes and leaves.

Although the plant performance is attributed to the genetic factors, differences in the biochemical parameters are of great importance, as the plant metabolism depends on various biochemical constituents. It is known that thousands of reactions are undergoing in the plants simultaneously which ultimately decide the growth and development and the final yield. Plant growth regulators have been shown to influence these processes in one way or the other.

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