THE EFFECTS OF *CANNABIS SATIVA* HYDROEXTRACTS ON *TRITICUM AESTIVUM* SEEDLINGS PIGMENT CONCENTRATIONS

Şolea Răzvan Mihai*, Şerban Georgeta**

**University of Oradea, Faculty of Medicine and Pharmacy, Doctoral School of Biomedical Sciences, Pharmacy Department, 29 Nicolae Jiga, 410028, Oradea, Romania, e-mail: razvanm1989@yahoo.com*
***University of Oradea, Faculty of Medicine and Pharmacy, Pharmacy Department, Pharmaceutical Chemistry, 29 Nicolae Jiga, 410028, Oradea, Romania, e-mail: getaserban_2000@yahoo.com*

Abstract

Cannabis, or hemp, is a plant with a huge medical and economic potential. Known since ancient times, Cannabis is rich in cannabinoids of which cannabidiol (CBD) is the most biologically active compound with almost none of the negative aspects of its fellow cannabinoids. Since the 19th century, Cannabis has been used as an analgesic, anesthetic, antidepressant, sedative and even antibiotic. However, its bio-industrial potential has not yet been fully explored and given that Cannabis plants are harvested for hemp, animal feed and various agricultural purposes, their potential effects as fertilizer or herbicide have not yet been identified.

This paper presents the effects of C. sativa hydroextract on Triticum aestivum seedlings. During this study it was observed that Triticum aestivum seedlings treated with 100%, 10% and 5% C. sativa extracts showed lower levels of Chlorophyll A and B than the control sample. The seedlings treated with 50% and 25% C. sativa extracts showed higher levels of Chlorophyll A and lower content of Chlorophyll B when compared to the control. There was an increase in the carotenoids detected in all samples, with 50% and 25% showing the highest levels. This suggests that C. sativa extracts have the ability to increase the carotenoids levels in T. aestivum seedlings and modify the amount of Chlorophyll A and Chlorophyll B extract in a dose-dependent manner.

Key words: *Cannabis sativa*, extract, spectrophotometry, carotenoids, chlorophyll, *Triticum aestivum*

INTRODUCTION

Cannabis, also known as marijuana, is the dried and processed leaves, flowers and body of the genus Cannabis. The oldest mention of it dates back to China in the year 2,120 B.C. (Devinsky et al., 2014; Russo, 2007). Cannabis contains substances called cannabinoids of which two cannabinoids, namely *Tetrahydrocannabinol* (THC) and *Cannabidiol* (CBD), are the ones with the highest level of medical potential. Nowadays there is an increase interest for CBD's and THC's analgesic, anti-nociceptive, anti-inflammatory, neuroprotective, immuno-stimulative, anti-cancerous and many more effects (Elias et al., 2019; Massi et al., 2004; Klein et al., 2003; Lah et al., 2021; Go et

al., 2020; Qamri et al., 2009), with particular interest towards CBD's medical potential. The reason why CBD has such an immense medical potential is that it does not trigger a "high" reaction as THC does.

The effects of CBD are mediated by the G protein coupled receptors, which are divided into two categories namely type I (CB1) and type II (CB2**)** (Jones et al., 2010). CB1 receptors are in abundance in the central nervous system, especially in the hippo-campus region and the CB2 receptors are primarily located in the immune system. Upon activation of CB1 receptors, an inhibition of synaptic transmissions was found through potassium and calcium channels, which are known to modulate epileptic and convulsive activity (Welty et al., 2014; Giacobbe et al., 2020).

CBD has multiple beneficial effects on the central nervous system therefore, in addition to its potential in the treatment of seizures, it reduces anxiety and sleep disorders (Shannon et al., 2019; Welty et al., 2014; Sholler et al., 2020).

The most recent area of interest for CBD is its potential use in the treatment of Covid-19-associated lung inflammation. Recent studies have shown that its anti-inflammatory and anxiolytic effects, combined with its ability to inhibit the "cytokine storm", gave rise to the idea of using it as an adjunct in the treatment of Covid-19 (Shi et al., 2020; Anil et al., 2021).

Applications of *Cannabis sativa* in areas such as the textile, agricultural or construction industries have recently been rediscovered and re-evaluated (Bailoni et al., 2021). Because the main focus is on its medical properties, the textile, industrial and agricultural potential are overlooked or completely ignored in current studies. This paper focuses on the effects of aqueous *C. sativa* extract on *Triticum aestivum* seedlings.

MATERIAL AND METHOD

The biological material consisted of carefully selected caryopsis of *Triticum aestivum* L., Trublion variety, C1, semi-rotten wheat, harvest year 2020. The *Cannabis sativa* (non-THC strain) was provided by Canah International Srl and Alcos Bioprod SRL.

To 1g of dried and grounded leaves were added 9g of distilled water and the mixture was kept at room temperature for 48 hours according to the Aguawa and Mittal extraction method (Aguawa et al., 1981). After filtration, the resulting extract has been labelled as 100% *C. sativa* hydroextract and kept at 4ºC until the

beginning of the experiment. Subsequent dilutions of 50%, 25%, 10%, 5%, 2.5% and 1%, respectively were made from the crude extract.

Ten germinated seeds of *Triticum aestivum* were placed on a filter paper in a Petri dish and 5 mL of *C. sativa* extract was added. The control was prepared by adding 5 mL of double distilled water. Each experiment was performed in triplicate for extracts of each concentration. The Petri dishes were kept for a period of 5 days for the roots and body of the infant grain plant to grow.

On the 5^{th} day, a weight of 0.05 g of plant (body and leaf) from each Petri dish was treated with a solution of 80% acetone. After a period of 72 hours, the mixture was decanted and the resulting solution was analyzed using a PG Instruments T80+ UV/VIS spectrometer. The readings for the samples were done at 470 nm, 646 nm and 663 nm wavelengths, along with the control for comparison. The absorption reading was performed seven times for each wavelength and then the arithmetic average was calculated.

Wellburn's formulas were used to determine the concentration of Chlorophyll A, Chlorophyll B and Carotenoids:

Chlorophyll A μg/mL = 11.65 x Abs 663 nm – 2.69 x Abs 646 nm

Chlorophyll B μg/mL = 20.81 x Abs 646 nm - 4.53 x Abs 663 nm

Carotenoids μg/mL = (1.000 x Abs 470 nm - 0.89 x Chlorophyll A - 52.02 x Chlorophyll B)/245

RESULTS AND DISCUSSION

As shown in Tables 1 and 2 and in Chart 1, the results showed a marked decrease in Chlorophyll B and an increase in Chlorophyll A and carotenoids, the 50% and 25% *C. sativa* extracts being the ones that produced the most significant results.

Chlorophyll A and B differ in their roles in the photosynthesis process, with Chlorophyll A being the main pigment that directly captures the light and Chlorophyll B being the accessory pigment with the role of collecting energy and passing it to Chlorophyll A. Chlorophyll A has the property to absorb light at 430 nm to 660 nm from the violet-blue and orange-red spectrums, while Chlorophyll B absorbs primarily blue spectrum of light at 450 nm to 650 nm.

Chlorophyll B has a higher presence in shade adapted chloroplasts, therefore one of Chlorophyll B's functions is absorbing energy in conditions of indirect sunlight/shaded areas and passing the energy to Chlorophyll A. An increase in the level of Chlorophyll A means that the ability of a plant to capture

light directly increases, while the decrease in Chlorophyll B means a decrease in the ability to indirectly capture light in shaded areas (Rabinowitch et al., 1965).

Carotenoids are pigments that have a dual role of photoprotective agents and have accessory properties for collecting light, along with which they give the plants a reddish to orange coloration. The hydroextracts of *C. sativa* showed the ability to increase the total amount of carotenoids in *T. aestivum* seedlings at each concentration studied. This imparts an increased or huge photoprotection in *T. aestivum* seedlings, the highest being at concentrations of 50% and 25%. Anyway, a marked increase was observed at all concentrations in terms of carotenoid levels.

Table 1

UV/Vis average absorption values of the samples at each wavelength

Sample	**Chlorophyll A 470 nm**	**Chlorophyll B 646 nm**	**Carotenoids 663 nm**
Control	0.698	0.803	0.612
100% *C. sativa*	0.489	0.198	0.235
50% *C. sativa*	0.924	0.475	0.892
25% *C. sativa*	1.103	0.638	1.073
10% *C. sativa*	0.530	0.310	0.461
5% *C. sativa*	0.376	0.214	0.244
2.5% *C. sativa*	0.822	0.442	0.719
1% *C. sativa*	0.607	0.352	0.504

Table 2

The content of Chlorophyll A, Chlorophyll B and carotenoids in the samples

Sample	**Chlorophyll A** [µg/mL]	**Chlorophyll B** [µg/mL]	**Carotenoids** [µg/mL]
Control	4.969	13.938	0.1284
100% *C. sativa*	2.207	3.060	1.338
50% *C. sativa*	9.113	5.844	2.497
25% *C. sativa*	10.784	8.332	2.693
10% *C. sativa*	4.5367	4.362	1.220
5% *C. sativa*	2.266	3.428	0.798
2.5 % *C. sativa*	7.187	5.940	1.825
1% *C. sativa*	4.924	5.042	1.389

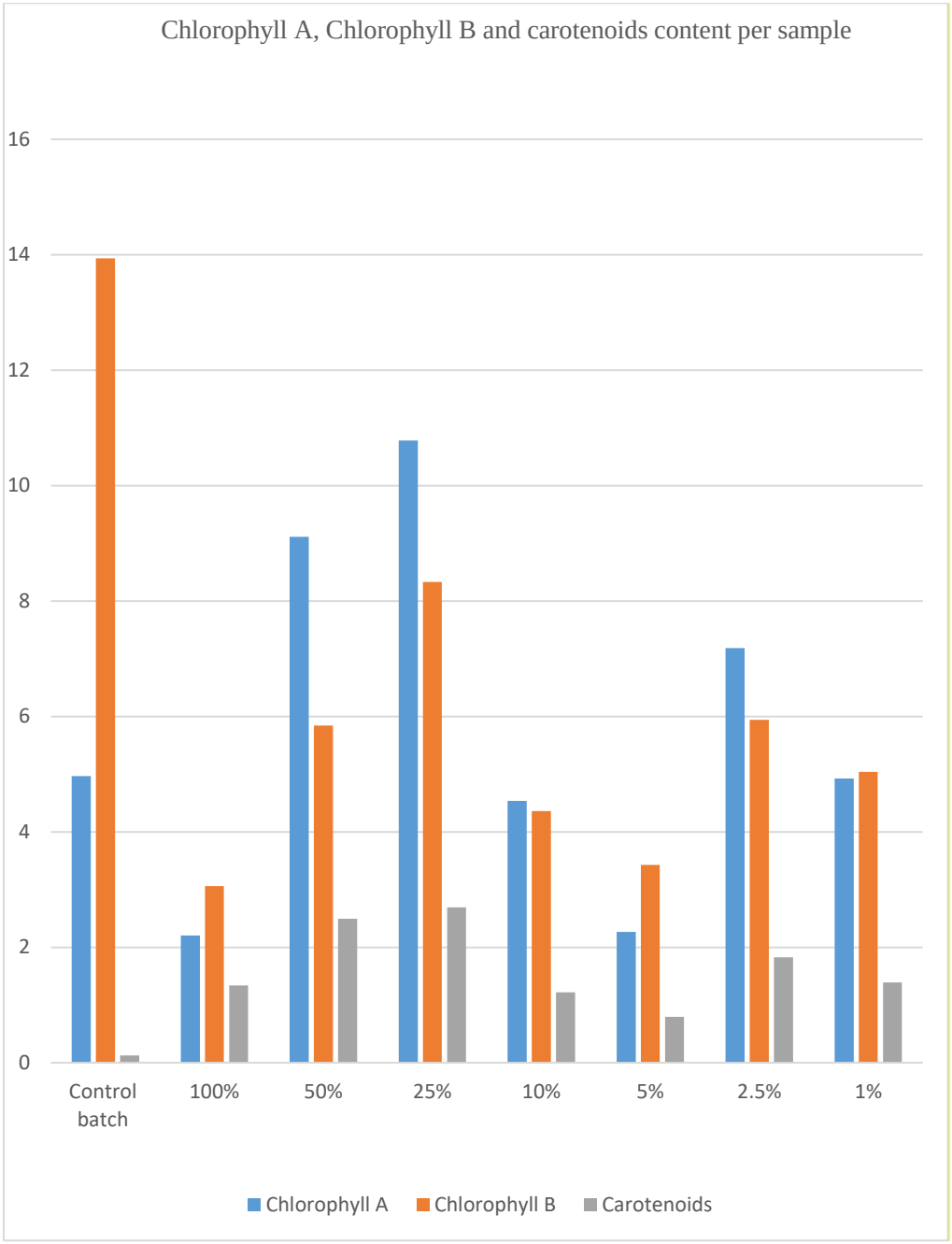

Fig. 1. Levels of Chlorophyll A, Chlorophyll B and carotenoids in *T. aestivum* seedlings treated with *C. sativa* hydroextracts

Taking into account these results it can be suggested that *C. sativa* might be used to create specialized crops for regions with inhospitable climates (e.g. higher temperatures, dry areas, higher intensity of sunlight) that are resistant to high levels of sunlight that would cause the standard crops to wither from too much light, as well as for obtaining crops with a high content of carotenoids for aesthetic and industrial purposes.

The data shows that the effects of *C. sativa* hydroextracts on *T. aestivum* seedlings are dose dependent. As suggested by Fig. 1, the level of Chlorophyll A detected in the samples treated with 50% and 25% *C. sativa* extracts, respectively shows that a specialized culture can be obtained which has a high capacity to directly absorb sunlight at wavelengths of 450 to 650 nm. This leads to the hypothesis of specialized crops that would be optimal for artificial lighting conditions with purple-blue and orange-red light in the range of 450 to 650 nm with direct constant light sources, such as greenhouses. On the other hand, the seeds treated with 10% and 1% *C. sativa* extracts suggest a plant adapted to equally absorb purple-blue and orange-red light by Chlorophyll A and blue by Chlorophyll B, with an equal ability to capture direct and indirect sunlight.

CONCLUSIONS

The effect of *C. sativa* hydroextracts on the levels of Chlorophyll A and B is dose-dependent. *T. aestivum* seedlings treated with 100%, 10% and 5% extracts, respectively showed lower levels of Chlorophyll A and B than the control, while the seedlings treated with 50% and 25% extracts showed high level of Chlorophyll A and low content of Chlorophyll B thus resulting in plants with a higher capacity to absorb direct sunlight and a reduced capacity to absorb indirect/shaded light.

This shows that, depending on the concentration of the extract, plants with a different content of chlorophyll and/or carotenoids pigments can be grown. With a significant increase in dose-dependent carotenoids and an increase in the photoprotection of plants, we can conclude that *C. sativa* extracts have an overall potential for designer crops that can be grown in areas with intense light, more intense than the natural climate of the plant. In addition, we can suggest that *C. sativa* hydroextract has the ability to create stronger and more specialized plants, but also to inhibit their development in a dose-dependent manner. This suggests the potential of *C. sativa* aqueous extract to be used to create crops that are tailor made for harsher lighting conditions or more artificial ones.

REFERENCES

1. Aguawa C.N., Mittal G.C., 1981, Study of antiulcer activity of aqueous extract of leaves of *Pyrenacanthia staudtii* using various models of experiment gastric ulcer in rats. European Journal of Pharmacology, 74, pp.215-220
2. Anil S.M., Shalev N., Vinayaka A., Nadarajan S., Namdar D., Belausov E., Shova I., Mani K.A., Mechrez G., Koltai H., 2021, Cannabis compounds exhibit anti-inflammatory activity in vitro in COVID-19-related inflammation in lung epithelial cells and pro-inflammatory activity in macrophages. Scientific Reports, 11, p. 1462-1475
3. Bailoni L., Bacchin E., Trocino A., Arango S., 2021, Hemp (*Cannabis sativa* L.) seed and co-products inclusion in diets for dairy ruminants: a review. Animals, 11, pp.856-869
4. Devinsky O., Cilio M.R., Cross H., Fernandez-Ruiz J., French J., Hill C., Katz R., Di Marzo V., Jutras-Aswad D., Notcutt W.G., Martinez-Orgado J., Robson P.J., Rohrback B.G., Thiele E., Whalley B., Friedman D., 2014, Cannabidiol: Pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia, 55, pp. 791-802
5. Elias R., Raheb M., Mekhaiel D., Cernovsky Z., Sidhu G., Warren D., Sadek G., Chiu S, Bureau Y., 2018, Use of pharmaceutical analgesics versus Cannabis or Cannabidiol-Tetrahydrocannabinol oils to reduce pain. Archives of Psychiatry and Behavioral Sciences, 1, pp.37-40
6. Giacobbe J., Marrocu A., Di Benedetto M.G., Pariante C.M., Borsini A., 2021, A systematic, integrative review of the effects of the endocannabinoid system on inflammation and neurogenesis in animal models of affective disorders. Brain Behavior and Immunity,93, pp.353-367
7. Go Y.Y., Kim S.R., Kim D.Y., Chae S.W., Song J.J., 2020. Cannabidiol enhances cytotoxicity of anti-cancer drugs in human head and neck squamous cell carcinoma. Scientific Reports, 10, 20622
8. Jones N.A., Hill A.J., Smith I., Bevan S.A., Williams C.M., Whalley B.J., Stephens G.J., 2010, Cannabidiol displays antiepileptiform and anti-seizure properties in vitro and in vivo. Journal of Pharmacology and Experimental Therapeutics, 332, pp.569-577
9. Klein T.W., Newton C., Larsen K., Lu L., Perkins I., Nong L., Friedman H., 2003, The cannabinoid system and immune modulation. Journal of Leukocyte Biology, 74, pp. 486-496
10. Lah T.T., Novak M., Almidon M.A.P., Marinelli O., Baskovic B.Z., Majc B., Mlinar M., Bosnjak R., Breznik B., Zomer R., Nabissi M., 2021. Cannabigerol is a potential therapeutic agent in a novel combined therapy for glioblastoma. Cells, 10, pp.340-361
11. Massi P., Vaccani A., Ceruti S., Colombo A., Abbracchio M.P., Parolaro D., 2004, Antitumor effects of cannabidiol, a nonpsychoactive cannabinoid, on human glioma cell lines. Journal of Pharmacology and Experimental Therapeutics, 308, pp.168-845

12. Qamri Z., Preet A., Nasser M.W., Bass C.E., Leone G., Barsky S.H., Ganju R.K., 2009, Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. Molecular Cancer Therapeutics, 8, pp.3117-3129
13. Rabinowitch E.I., Govindjee G., 1965, The role of Chlorophyll in photosynthesis. ScientificAmerican, 213, pp.74-83
14. Russo E.B., 2007, History of cannabis and its preparations in saga, science, and sobriquet. Chemistry and Biodiversity, 4, pp.1614-1648
15. Shannon S., Lewis N., Lee H., Hughes S., 2019, Cannabidiol in anxiety and sleep: a large case series. The Permanente Journal, 23, 18-041
16. Shi Y., Wang Y., Shao C., Huang J., Gan J., Huang X., Bucci E., Piacentini M., Ippolito G., Melino G., 2020, COVID-19 infection: the perspectives on immune responses. Cell Death and Differentiation, 27, pp.1451-1454
17. Sholler D.J., Schoene L., Spindle T.R., 2020, Therapeutic efficacy of Cannabidiol (CBD): a review of the evidence from clinical trials and human laboratory studies. Current Addiction Reports, **7**, pp.405-412
18. Welty T.E., Luebke A., Gidal B.E., 2014, Cannabidiol: promise and pitfalls. Epilepsy Currents, 14, pp. 250-252