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Studies regarding treatments of LED-s emitted light on sprouting hemp (*Cannabis sativa* L.)

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Abstract
The purpose of our paper was to evaluate the influence exercised by LED-s emitted light and sunlight treatments on the hemp (Cannabis sativa L.), sprouts. The sprouts were illuminated with three types of LED-s spectrum variants red (R), blue (B) and green (G), and the sunlight represented the reference. Our results showed that blue (B) light LED-s determined a high level of rate and fresh weight of hemp sprouts. The phytochemical compounds content (polyphenols, flavonoids), antioxidant activity (determined by DPPH assay), and protein concentration were evaluated in hemp (Cannabis sativa L.), sprouts. The content of polyphenols, flavonoids and protein was significantly higher by treatment with blue (B) light LED-s than sunlight while green (G) light LED-s improved the antioxidant capacity.

Key words: *Cannabis sativa* L., sprouts, LED-s, polyphenols, antioxidant capacity, proteins, flavonoids

Introduction

Recently the food research has focused on the identification of functional foods which contain biologically active molecules that can make benefits to health, beyond the nutritional role (S. FRASSINETTI & al., [1]). In this respect, the obtaining of sprouts is considered one of the processing methods that enhanced the nutritive value and the health qualities of foods in a natural way. This method has been known for a very long time in China and Japan. The sprouting is simple and cheap method and different seeds can be sprouted for human consumption: legumes (bean, pea, lentil, soybean), grains (rye, wheat, barley, oats) and more recently, seeds of many vegetables (alfalfa, radish) and also types of seeds with economic importance, like hemp (*Cannabis sativa* L.).
Cannabis sativa L. is an annual species belongs to the *Cannabis* Genus, *Cannabaceae* Family (http://www.theplantlist.org/tpl1.1/record/kew-448365 [2]), *Urticales* Order, *Dicotyledonae* Class, *Angiospermae* Subphylum, *Spermatophyta* Phylum, *Plantae* Kingdom and *Eukaryota* Domain (https://www.cabi.org/isc/datasheet/14497 [3]), which is cultivated in the northern and southern hemisphere (https://www.cabi.org/isc/datasheet/14497 [3]; https://www.reportlinker.com/p04143755/Global-Hemp-Based-Foods-Market.html [4]).
Cannabis sativa L. is a species with many uses, from industrial products (e. g., textiles, foods, paper, building material, plastic and composite materials for automotive and aviation sectors – R. JOHNSON, [5]), to nutritional supplements, bio-pharmacologic (R. JOHNSON, [5]), and therapeutic properties (e. g., glaucoma - T. JÄRVINEN & al., [6], anxiety - S. TAMBARO & M. BORTOLATO, [7], alleviation of symptoms of chemotherapy for cancer - N. E. SLATKIN, [8]; S. GIACOPPO & al.,[9] and multiple sclerosis symptoms - S. GIACOPPO & al., [9]).
The seeds of hemp (*Cannabis sativa* L.), contain many usefull elements for human nutrition (carbohydrates, polyunsaturated fats, proteins, vitamins and minerals). Informations on the internet for cultivation of hemp (*Cannabis sativa* L.), are many from open space (under sunlight), and few to close

space (under light LED-s irradiation). But, we are not found, in scientific literature reading, study for obtaining of sprouts under light LED-s irradiation and in aseptically conditions.
The LED-s have many advantages over neon light sources (e. g., much less power consumption (http://www.lighting.philips.com/main/support/support/faqs/general-questions/applications/what-are-the-advantages-of-led# [10]).
In this context, the objective of our studies was to establish the influence exercised of treatments with LED-s emitted light (red, blue or green) and sunlight on the rate, the fresh weight, the proteins, polyphenol, flavonoids concentration and antioxidant activity of hemp (*Cannabis sativa* L.), sprouts.

Materials and Methods
The **biological material,** was represented by *Cannabis sativa* L. subsp. *indica* seeds, in their dormant phase. The seeds have been obtained from S. C. Hofigal Export-Import S. A. . The seeds germination and sprouts were occurred under sterile conditions. The seeds sterilization has been carried out according to the following protocol: 1 minute immersion into a 70% $C_6H_{12}O_6$ solution and three times washing with sterile distilled water (E.M. BADEA & D. SĂNDULESCU, [11]; D. CACHIȚĂ-COSMA & al., [12]). The seeds inoculation was accomplished in recipients on sterile gauze soaked in 10 ml sterilized distilled H_2O (I.M. ENACHE & O. LIVADARIU, [13]), under dark conditions for 72 hours.
The light – based treatments were performed using four types of irradiation, respectively: sunlight, and the LED-s irradiated light, with three spectrum variants (deep red - C. DONG & al., [14], high blue, and green). The treatments with sunlight or LED-s irradiated light have been applied during the 16 h photoperiod, for 4 days, with incubation at a temperature of 23°C ± 2°C / photoperiod and at the temperature of 20°C ± 2°C / dark period. The technical characteristics of LED-s are: voltage 220 V, power 18 W and light flux 435 lm (O. LIVADARIU & C. MAXIMILIAN [15]).
The experimental plan consisted of four experimental variants:
- V1 / S = treatment by sunlight (S) - control;
- V2 / R = treatment by red color LED-s – emitted light (R);
- V3 / B = treatment by blue color LED-s – emitted light (B)
- V4 / G = treatment by green color LED-s – emitted light (G).

Statistical procedures. The variants were consisted from 15 hemp seeds. All analysis were performed in triplicate. The data have been statistically analysed and the standard deviation of mean was calculated. The rate, the fresh weight of sprouts (**A**), antioxidant activity, polyphenols, flavonoids, proteins content (**B**) were achieved.
1. **The protein extraction** was performed by grinding the sprouts tissue in 50 mM potassium phosphate buffer, 0,05% β-mercaptoethanol, 0,5 mM (DIFP) diizopropil fluorophosphat –protease inhibitor, pH = 6.8, (1 g / 0.5 ml, dry weight / buffer) at 4°C for 24 hours. The extract was centrifuged 18.000 rpm for 20 min and the supernatant was used for protein assay. The protein concentration was carried out using Bradford method (M.M. BRADFORD, [16]), based on binding of protein by Coomassie Blue and measurement the absorbance of protein-dye complex at 595 nm.
2. **Preparation of methanolic extracts**. 4 ml of 100% methanol were added to 1g of sprout and grounded in a mortar with pestle. The extract was maintained overnight to 4^{o}C. After centrifugation 20 min. at 15.000 rpm the supernatant was used for determination of phenolic compounds, antioxidant capacity and flavanoids content.
3. **The protein concentration** was carried out using Bradford method (1976) based on binding of protein by Coomassie Blue and measurement the absorbance of protein-dye complex at 595 nm.
4. **The polyphenol content** in methanolic extracts was evaluated using a modified method with Folin-Ciocalteu reagent (V. MIHAILOVIĆ & al., [17]). The reaction mixture consisted from 0.5 ml methanolic extract, 2.5 ml Folin-Ciocalteu diluted 1:10 and 2 ml 7.5% Na_2CO_3. The mixture was incubated for 30 min. at room temperature. The absorbance was measured at 765 nm. The calibration curve was prepared with different concentrations of gallic acid. The results are expressed in mg equivalent gallic acid /g fresh weight.

5. **The antioxidant capacity** of methanolic extracts was carried out according to Marxen (K. MARXEN & al., [18]), using DPPH (2,2-diphenyl-1-picrylhydrazyl) and a calibration curve with Trolox as antioxidant standard. The mixture was incubated at room temperature for 30 min. and spectrophotometrically detected at 517nm. The antioxidant capacity was expressed in μMTrolox/g fresh weight.

6. The flavonoid compounds in methanolic extracts were estimated using Zhishen (J. ZHISHEN & al., [19]), modified method with aluminum chloride. The 0.5 mL of methanolic extract were mixed with 2ml of distilled water and 150 μl of 5% sodium nitrate. After 5 min., added 150 μl of 10% aluminum chloride and incubated for 6 min and then 2ml of 4% sodium hydroxide were added. Absorbance of the mixtures was measured at 510 nm. It used a calibration curve with rutin. The flavonoids concentration was expressed in mg equivalent rutin / g fresh weight.

Results and Discussions

A. Determination of the rate and the fresh weight of hemp (*Cannabis sativa* L.) sprouts by irradiation with red (R), blue (B) and green (G) LED-s and sunlight

A1. Determination of **the rate** of *Cannabis sativa* L. sprouts (**Figure 1**), showed the highest value for variants V3 / B and the lowest value in the case of variant V1 / S. The values of the rate from others two sprout variants (V2 / R and V4 / G), has been similarly.

Thus, the treatment with blue LED (V3 / B), induced a superior rate of sprouts in comparison with others variants. The R, B or G LED-s treatments determined a higher rate sprouts in comparison with sunlight.

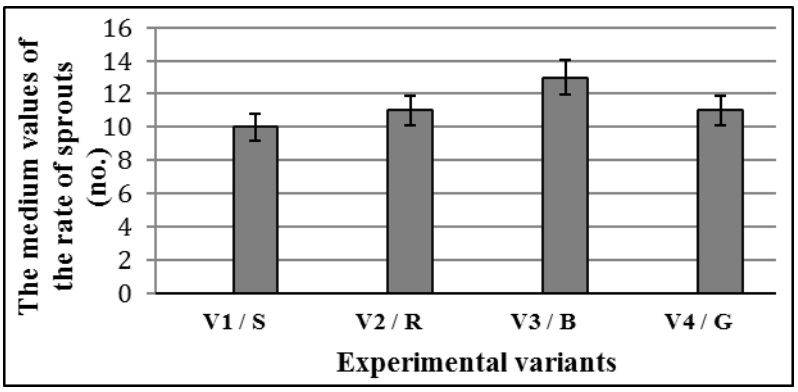

Figure 1. The medium values of **the rate** of *Cannabis sativa* L. sprouts (no.), for experimental variant (V1-V4)

A.2. Determination of **the fresh weight** of *Cannabis sativa* L. sprouts (**Figure 2**), showed that the highest value for variant V3 / B (0.608 g), and the lowest value for variant V1 / S (0.519 g).

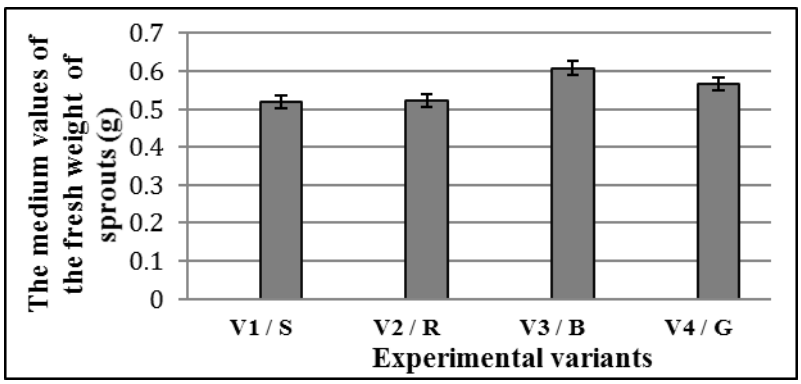

Figure 2. The medium values of **the fresh weight** of *Cannabis sativa* L. sprouts (g), for experimental variant (V1-V4)

The comparable values were obtained in the case of variants V1 / S (0.519 g), and V2 / R (0.522 g).
The analysis of two sources of light (sunlight or light LED-s irradiation) demonstrated that the sunlight (S) and red (R) LED-s irradiated light determined the comparable fresh weight of sprouts, but green (G) and blue (B) LED-s irradiated light determined a high value of the fresh weight of sprouts.

B. Biochemical analyses

B.1. **The proteins concentration** of *Cannabis sativa* L. sprouts illuminated with green, blue, red LED-s and sunlight **(Figure 3)**

The illumination with blue light LED (V3/B) produced the highest protein concentration for hemp sprouts. The sunlight illumination induced a protein concentration lower than blue light LED but higher than green (V4/G) and red (V2/R) LED. The lowest protein concentration was determined by red light LED treatment. These results are similar with our previous experiment accomplished on wheat sprouts, S2 variety (D. RAICIU & al., [20] - in press).

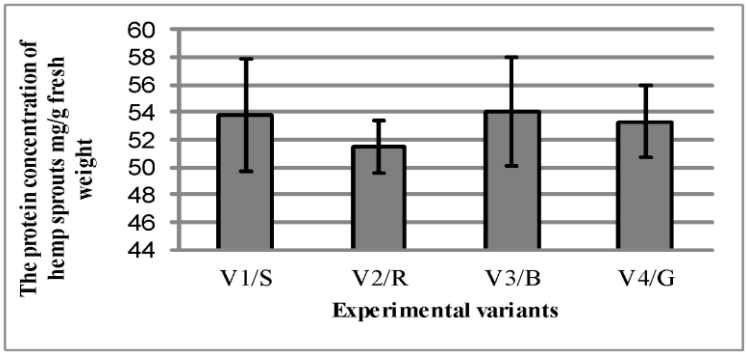

Figure 3. The **proteins concentration** of *Cannabis sativa* L. sprouts for experimental variant (V1-V4)

B.2. **The antioxidant capacity** of *Cannabis sativa* L. sprouts illuminated with green, blue, red LED-s or sunlight **(Figure 4).**

The antioxidant capacity in hemp sprouts was higher by treatment with green illuminated LED. The sunlight induced the lowest response of antioxidant capacity, while red light LED (V2/R) treatment produced a higher value in comparison with blue light LED (V3/B).

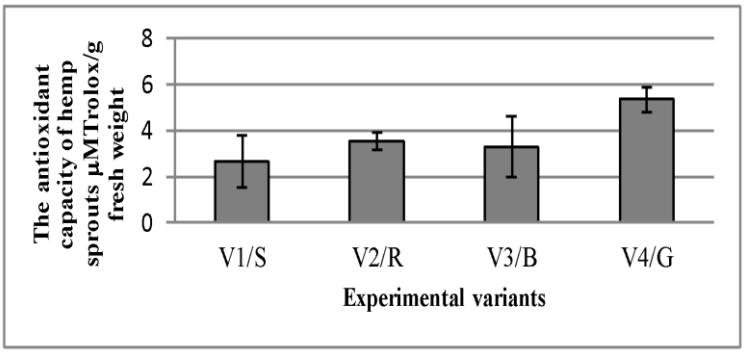

Figure 4. The **antioxidant capacity** of *Cannabis sativa* L. sprouts for experimental variant (V1-V4)

B.3.The **polyphenols concentration** of *Cannabis sativa* L. sprouts illuminated with green, blue, red LED-s or sunlight **(Figure 5)**

The blue (B) light LED induced the highest polyphenols concentration in hemp sprouts. The green light LED determined the lowest concentration of polyphenols while sunlight induced a higher content than illumination with red (R) light LED.

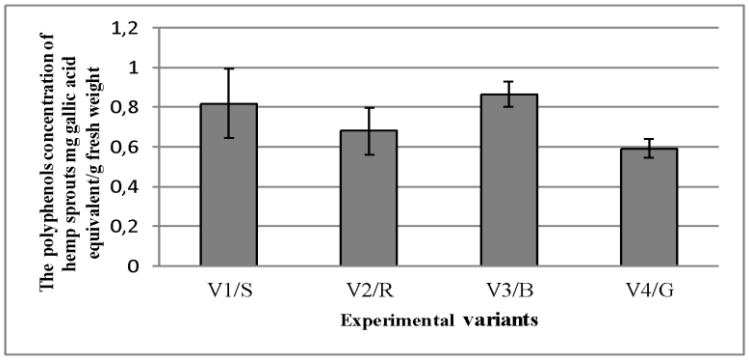

Figure 5. The **polyphenols concentration** of *Cannabis sativa* L. sprouts for experimental variant (V1-V4)

O. LIVADARIU & C. MAXIMILIAN [15], reported that the polyphenol content of sprouts buckwheat (sprouting from seeds with testa) illuminated with blue LED was higher in comparison with treatment with white and red light LED .

B.4. The **flavonoids concentration** of *Cannabis sativa* L. sprouts illuminated with green, blue, red LED-s or sunlight **(Figure 6)**
The highest concentration of flavonoids in hemp sprouts was determined by illumination with blue (B) LED light while the lowest value was registered on green (G) light LED treatment. The sunlight exposition caused a higher flavonoids content than illumination with red (R) light LED.

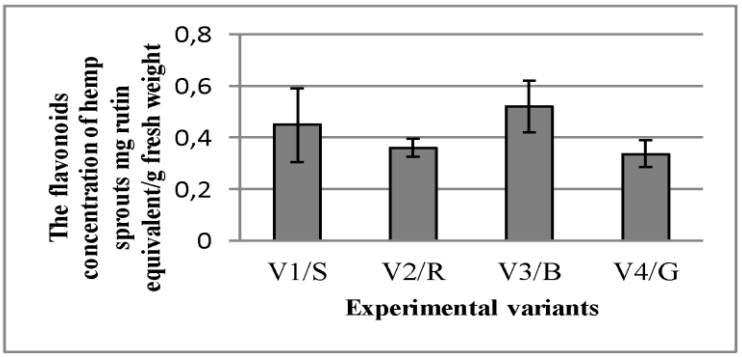

Figure 6. **The flavonoids concentration** of *Cannabis sativa* L. sprouts for experimental variant (V1-V4)

Conclusions
These results lead to conclusion that source of light (sunlight or light LED-s irradiation) and color of light LED-s influences the number of sprouts rate which can be achieved.
The growth characteristic, such fresh weight and the sprouting promotion was the most effective under the LED treatments compared with the control (sunlight).
The studies regarding the influence exercised of treatments with LED-s emitted light (red, blue or green) and sunlight on the rate, the fresh weight, the proteins content, polyphenols and flavonoids concentration and antioxidant activity of *Cannabis sativa* L. sprouts, demonstrate that: **the rate of sprouts** illuminated with LED-s was higher than control represented by sunlight; **the fresh weight of sprouts** was superior in case of illumination with the LED-s in comparison with the sunlight treatment; **the rate of sprouts** and **the fresh weight of sprouts** were improved by illumination with LED-s emitted blue light (V3 / B), and the treatments with blue (B) light LED-s determined the amplifying of the metabolic pathways for biosynthesis of **proteins** and **flavonoids**, **polyphenols**. The green (G) light LED treatment produced an increasing of **antioxidant capacity**.

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