

The evaluation of the effect of LED-s irradiation on wheat sprouts (*Triticum aestivum* L.)

Received for publication, December, 2, 2017

Accepted, January, 12, 2018

DANIELA RAICIU^{1,4*}, OANA LIVADARIU², CARMEN MAXIMILIAN³, ADRIANA BIRA⁴

¹University of "TITU MAIORESCU", Faculty of Pharmacy Department of Pharmacognosy, Phytochemistry, Phytoterapy,, Gh. Sincai Street no.16, 4 district, Bucharest, Romania

²Faculty of Biotechnologies, UASVMB, 59 Bd. Marasti, 011464, Bucharest, Romania

³Institute of Biology Bucharest, Romanian Academy, 296 Spl. Independentei, 060031, Bucharest, Romania

⁴S.C.Hofigal Export-Import S.A., Entrance of Sere Street, no.2, 4 district, Bucharest, Romania

*Address for correspondence to: daniela_raiciu@yahoo.com

Abstract

Wheat sprouts (*Triticum aestivum* L.), are recommended for human food consumption, due to the rich content in nutritional elements of high level bioavailability. This particular bioavailability is given by genetic information and the capacity of wheat seeds (*Triticum aestivum* L.), to convert light energy into biochemical energy. The objectives of our work was to assess the effect exercised by LED-s and sunlight treatments on the wheat sprouts in terms of the number, fresh weight, polyphenols concentration, proteins and flavonoids content, as well as antioxidant activity. The best results obtained for the rate and the fresh weight of sprouts, have been achieved in the case of irradiation with red (R) light treatment. The influence exercised by LED-s emitted red (R) light improved the flavonoids concentration. The highest concentration of polyphenols of wheat sprouts was determined by irradiation with sunlight, while the enhanced antioxidant activity was induced by illumination with blue (B) light LED. The higher proteins content was observed by red (R) illuminated LED.

Key words: wheat sprouts, LED-s, proteins, antioxidants

1. Introduction

Wheat (*Triticum aestivum* L.), is an annual species belongs to the *Triticum* Genus, *Poaceae* Family (<http://www.theplantlist.org/tp1.1/record/kew-448365>, [1]), which is cultivated at a global level in temperate climate areas (Europe, North and South America, Asia and Australia).

Wheat (*Triticum aestivum* L.), is a species having important contribution (R. J. PEÑA, [2]), to the human existence, being used for: human nutrition (flour, breads, cakes, drink, sprouts, etc. – (V. PATHAK & S. SHRIVASTAV, [3]), bio-pharmacology (antiinflammation and antioxidant activities, treatment of obesity, preventing rashes, etc. – (V. PATHAK & S. SHRIVASTAV, [3]), bio-degradable manufactures industries (textiles, wood construction materials, biodegradable plastic eating tools – (V. PATHAK & S. SHRIVASTAV, [3]), as well as necessary food for future air–spatial missions which are to be extended for a long period of time (C. DONG & al., [4]; B. LI & al., [5]). The nutritional quality of wheat (*Triticum aestivum* L.) derives from the contents in: good quality proteins, a high content of non–saturated fats, food fibers, minerals, vitamins as well as bioactive compounds (L. PLAZA & al., [6]; L. ALVAREZ-JUBETE & al., [7]; V. PATHAK & S. SHRIVASTAV, [3]).

The LED - irradiated light is a physical factor which can brings a decisive contribution to the obtaining of large biomass production. LEDs have multiple advantages over traditional light

sources: ability to emit a narrow band of light, high purity and efficacy, tiny size, longer half-life, and lower power consumption (S. ASTOLFI & al., [8]; F. XU & al., [9]).

Many studies from the last years are focused on the influence of LED-s irradiated light on the germination of seeds and the quality of germinated seed (antioxidant properties (G. SAMOULIENE & al., [10]), especially on wheat sprouts and the quality of plants being achieved (C. DONG & al., [4]).

The light emitting diode (LED) radiation can increase the antioxidant activity in pea seedlings (M.C. WU & al., [11]) and the phenolic content in buckwheat sprouts (Z. HOSEN, [12]).

The use of single-spectral blue or red LEDs has resulted in significant improvements in the quality and yield of vegetables and fruits (e.g., cucumber, pepper, and strawberry fruits) when compared with white fluorescent or solar light (H.G. CHOI & al., [13]; X. HAO & al., [14]; H.-M. LI & al., [15]).

In this respect, the aim of this paper is to evaluate the influence of treatments with LED-s illumination (red, white or blue) and sunlight on the rate, the fresh weight, the proteins, polyphenol, flavonoids concentration and antioxidant activity of wheat sprouts.

2. Materials and Methods

The **biological material** was represented by wheat seeds. The seeds have been obtained from two commercial sources (variety S1 and variety S2). The seeds germination and sprouts was occurred under sterile conditions. The seeds sterilization has been carried out according to the following protocol: 1 minute immersion into a 70% ethylic alcohol solution and three times washing with sterile distilled water (E.M. BADEA & D. SĂNDULESCU. [16]; D. CACHIȚĂ-COSMA & al., [17]). The seeds inoculation was accomplished in transparent recipients on sterile gauze soaked in 10 ml sterilized distilled water (I.M. ENACHE & O. LIVADARIU, [18]), under dark conditions for 24 hours.

The light – based treatments were performed using four types of irradiation, respectively: sunlight and the LED-s irradiated light, with three spectrum variants (cold white, deep red - C. DONG & al., [19] - and high blue). The treatments with sunlight or LED-s irradiated light have been applied during the 16 h photoperiod (I.M. ENACHE & O. LIVADARIU, [18]), for four days, with incubation at a temperature of $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / photoperiod and at the temperature of $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ / dark period (I.M. ENACHE & O. LIVADARIU, [18]). The technical characteristics of LED-s are: power 18 W, voltage 220 V and light flux 435 lm.

The experimental plan consisted of eight experimental variants:

- V1 / S1N = S1 + treatment by sunlight (N);
- V2 / S1W = S1 + treatment by white color LED-s – emitted light (W);
- V3 / S1R = S1 + treatment by red color LED-s – emitted light (R);
- V4 / S1B = S1 + treatment by blue color LED-s – emitted light (B);
- V5 / S2 N = S2 + treatment by sunlight (N);
- V6 / S2W = S2 + treatment by white color LED-s – emitted light (W);
- V7 / S2R = S2 + treatment by red color LED-s – emitted light (R);
- V8 / S2B = S2 + treatment by blue color LED-s – emitted light (B).

1. **The protein extraction** was performed by grinding the sprouts tissue in 50 mM potassium phosphate buffer, pH 7.2 mM EDTA, pH = 6.8, 4% pvp (polyvinyl pyrrolidone), (in relation to 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 estimation. The protein concentration was carried out using Bradford method (M.M. BRADFORD, [20]) 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°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 polyphenol content** in methanolic extracts was evaluated using a modified method with Folin-Ciocalteu reagent (V. MIHAILOVIĆ & al., [21]). The reaction mixture consisted from 0.5 ml methanolic extract, 2.5 ml Folin-Ciocalteu diluted 1:10 and 2 ml 7.5% Na₂CO₃. 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.

4. **The antioxidant capacity** of methanolic extracts was carried out according to Marxen et al. (K. MARXEN & al., [22]), 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.

5. **The flavonoid compounds** in methanolic extracts were estimated using Zhishen et al. (J. ZHISHEN & al., [23]), 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.

Statistical procedures. The variants were consisted from 15 wheat 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.

3. Results and Discussion

A. Determination of the rate and the fresh weight of wheat sprouts by irradiation with white, blue, red LED-s and sunlight

A.1. Determination of **rate of wheat sprouts** (Figure 1), showed the highest value in the case of variants V3 / S1R (14) and V6 / S2W (14), and the lowest value in the case of variant V2 / S1W (12). The values of the rate from others five sprout variants (V1 / S1N, V4 / S1B, V5 / S2N, V7 / S2R and V8 / S2B), has been similarly (13).

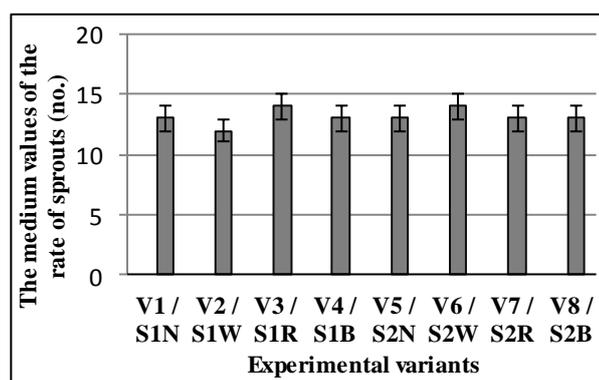


Figure 1. The medium values of **the rate** of wheat sprouts (no.),
for experimental variant (V1-V8)

Thus, the treatment with red LED (V3 / S1R) or with white LED (V6 / S2W) induced a superior rate of sprouts in comparison to the others variants. The red light (R) irradiated by LEDs determined a higher rate sprouts for S1 in comparison with variety S2.

The treatment applied with LED-s emitted white light (W) is more beneficial for variety S2 (V2/ S1W) as compared to variety S1 (V6/ S2W), in terms of sprouts rate. These results lead to conclusion that white (W) or red (R) light LED influences the type of wheat species will be used for achieved a large sprouts number.

A.2. Determination of **the fresh weight of wheat sprouts (Figure 2)**, showed that the highest value for variant V3 / S1R (2.12 g), and the lowest value for variant V7 / S2R (1.72g g).

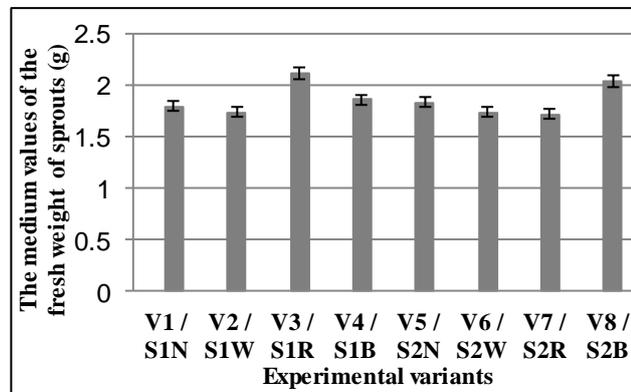


Figure 2. The medium values of **the fresh weight** of wheat sprouts (g),
for experimental variant (V1-V8)

The comparable values were obtained in the case of variants V4 / S1B (1.86 g), V5 / S2N (1.84 g) and V1/S1N (1.80 g). Similar values were observed for the variants V2 / S1W and V6 / S2W (1.74 g).

The analysis of two varieties (S1 and S2) demonstrated that the red (R) LED-s irradiated light determined the highest fresh weight of sprouts for variety S1 and a low value for variety S2. The sunlight (N) and white (W) LED-s irradiated light produced similar effect on the sprouts fresh weight for both varieties S1 and S2. The LED-s irradiated blue (B) light improved the sprouts fresh of variety S2 in comparison with variety S1.

B. Biochemical analyses

B.1. The proteins concentration of wheat sprouts illuminated with white, blue, red LED-s and sunlight (Figure 3)

The illumination with red light illuminated LED produced the highest protein concentration for S1 variety. The treatment with blue illuminated LED induced the best protein concentration for the S2 variety. If compared the impact of white illuminated LED for both variants of sprouts wheat it can be observe that the proteins concentration is higher to first variant S1. The red and blue light illuminate LED induced an opposite response to S1 and S2 varieties.

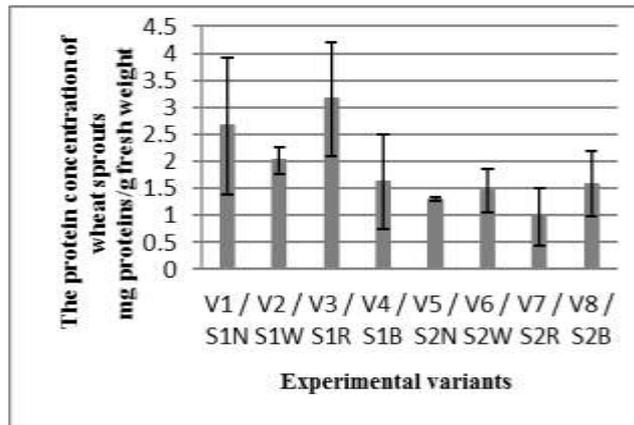


Figure 3. The proteins concentration of wheat sprouts for experimental variant (V1-V8)

B.2. The antioxidant capacity of wheat sprouts illuminated with white, blue, red LED-s or sunlight (**Figure 4**)

The antioxidant capacity in wheat sprouts was higher by treatment with blue illuminated LED for both S1 and S2 varieties. This result is similar to our previous studies (O. LIVADARIU & C. MAXIMILIAN, [24]), concerning the effect of blue LED light on buckwheat sprouts. The blue LED induced an increasing of antioxidant capacity on buckwheat sprouts. The red light LED induced the lowest proteins concentration for variety S1 while white LED light determined similar response for both varieties (S1 and S2).

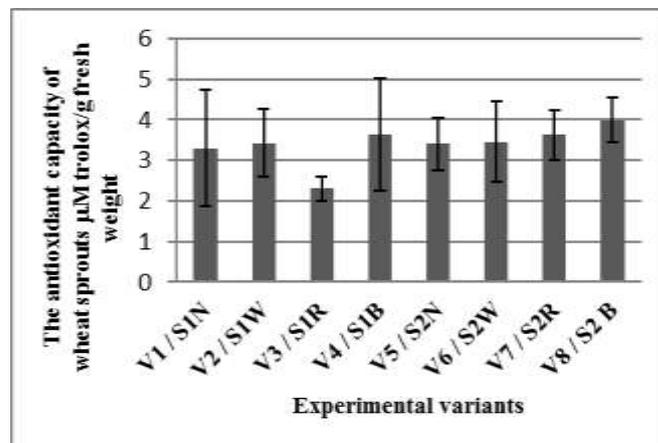


Figure 4. The antioxidant capacity of wheat sprouts for experimental variant (V1-V8)

B.3. The polyphenols concentration of wheat sprouts illuminated with white, blue, red LED-s or sunlight (**Figure 5**)

The sunlight induced the highest polyphenols concentration. The red illuminated LED produced very close values of polyphenols concentration for S1 and S2 varieties. The treatment with white LED determined twice as high concentration as that for S1 and S2 varieties.

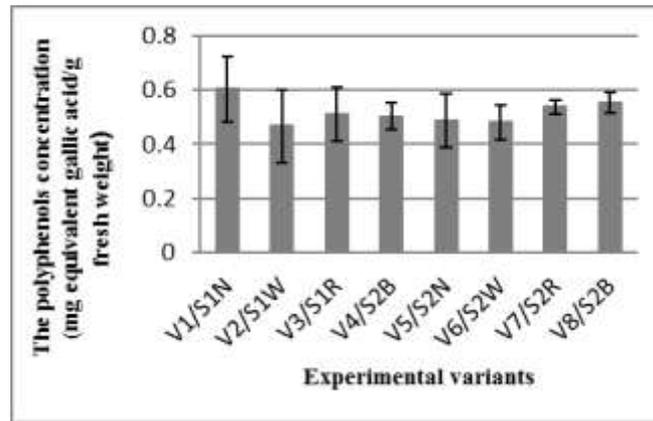


Figure 5. The polyphenols concentration of wheat sprouts for experimental variant (V1-V8)

B.4. The flavonoids concentration of wheat sprouts illuminated with white, blue, red LED-s or sunlight (Figure 6)

The highest concentration of flavonoids in wheat sprouts was determined by illumination with red LED light while the white LED light was similar for both S1 and S2 varieties but higher than the variant illuminated with sunlight. The red LED light produced an opposite effect for S1 variety (lowest value) and S2 variety (higher value).

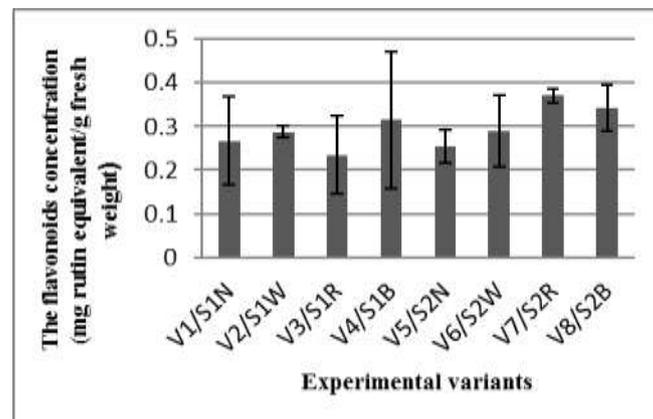


Figure 6. The flavonoids concentration of wheat sprouts for experimental variant (V1-V8)

Our previous studies (O. LIVADARIU & C. MAXIMILIAN, [24]), on sprouts buckwheat have shown similar results regarding the illumination with red and blue LED-s light. This species, considered a pseudocereal, contains the highest amount of flavonoids when is illuminated with red LED light.

4. Conclusions

The rate and the fresh weight, phenolic and flavonoids compounds, proteins and antioxidant activity were determined in the wheat sprouts cultivated under different LED lamps with different three spectrum variants (white, red and blue) and sunlight. The studies regarding the effect exercised by LED-s and sunlight treatments on wheat sprouts (*Triticum aestivum* L.), proved that:

- the LED treatment exercise a low level effect, similar or minor differences, over the relevant **rate of sprouts;**

- the LED treatments exercise a variable influence or minor differences in terms of **the fresh weight of sprouts**;
- irradiation with LED-s emitted red light (V3 / S1R) improved **the rate of sprouts and the fresh weight of sprouts**,
- the type of wheat varieties (S1 or S2) is relevant to obtained large number of sprouts to treatment with white or red LED-s irradiated light;
- the treatments with red LED-s light determined the amplifying of the metabolic pathways for biosynthesis of proteins and flavonoids;
- the highest concentration of polyphenols was obtained by illumination with sunlight;
- the highest antioxidant capacity was determined by illumination with blue LED light.

References

1. <http://www.theplantlist.org/tpl1.1/record/kew-448365>. The Plant List. A working list of all plant species.
2. R. J. PEÑA. Wheat for bread and other foods. BREAD WHEAT Improvement and Production, Ed. by Curtis B. C., Rajaram S., Gómez, H., *FAO Plant Production and Protection Series*, FAO, Rome, No. 30, 2002. (<http://www.fao.org/docrep/006/y4011e/y4011e0w.htm>).
3. V. PATHAK, S. SHRIVASTAV. Biochemical studies on wheat (*Triticum aestivum* L.). *Journal of Pharmacognosy and Phytochemistry*. 4: 3, pp. 171, 172, 175 (2015). (<http://www.phytojournal.com/archives/2015/vol4issue3/PartC/4-3-32.pdf>).
4. C. DONG, Y. FU, G. Liu, H. Liu. Growth, Photosynthetic Characteristics, Antioxidant Capacity and Biomass Yield and Quality of Wheat (*Triticum aestivum* L.) Exposed to LED Light Sources with Different Spectra Combinations. *J. Agro. Crop. Sci.*, 200: 3, pp. 219, 228 (2014). (<http://bme.buaa.edu.cn/UpFile/20150504175012.pdf>). DOI: 10.1111/jac.12059
5. B. LI, C. DONG, Z. CHU, W. ZHANG, M. WANG, H. LIU, B. XIE. Synthesis, characterization and application of ion exchange resin as a slow-release fertilizer for wheat cultivation in space. *Acta Astronautica*, 127, pp. 579 (2016). (<http://www.sciencedirect.com/science/article/pii/S0094576516302260>). <https://doi.org/10.1016/j.actaastro.2016.06.048>
6. L. PLAZA, B. DE ANCOS, P.M. CANO. Nutritional and health-related compounds in sprouts and seeds of soybean (*Glycine max*), wheat (*Triticum aestivum*.L) and alfalfa (*Medicago sativa*) treated by a new drying method. *Eur Food Res Technol*. 216: 2, pp. 138 (2003). (<https://link.springer.com/article/10.1007/s00217-002-0640-9>). DOI 10.1007/s00217-002-0640-9.
7. L. ALVAREZ-JUBETE, H. WIJNGAARD, E.K. ARENDT, E. GALLAGHER. Polyphenol composition and *in vitro* antioxidant activity of amaranth, quinoa, buckwheat and wheat as affected by sprouting and baking. *Food Chemistry*, 119, pp. 770 (2010). (<http://quinua.pe/wp-content/uploads/2013/02/antiox-Gallagher.pdf>).
8. S. ASTOLFI, C. MARIANELLO, S. GREGO, R. BELLAROSA. Preliminary investigation of LED lighting as growth light for seedlings from different tree species in growth chambers. *Not. Bot. Horti Agrobot. Cluj-Napoca*, 40, pp. 31–38 (2012).
9. F. XU, S.F. CAO, L.Y. SHI, W. CHEN, X.G. SU, Z.F. YANG. Blue light irradiation affects anthocyanin content and enzyme activities involved in postharvest strawberry fruit. *J. Agric. Food Chem.*, 62, pp. 4778–4783 (2014).
10. G. SAMOULIENE, A. VIRŠILĖ, A. BRAZAITYTĖ, G. ŠABAJEVIENĖ, J. SAKALAUŠKAITĖ, P. DUCHOVSKIS. The impact of LED illumination on antioxidant properties of sprouted seeds. *Central European Journal of Biology*, 6: 1, pp.68 (2011). (<https://link.springer.com/article/10.2478/s11535-010-0094-1>). DOI: 10.2478/s11535-010-0094-1
11. M.C. WU, C.Y. HOU, C.M. JIANG, Y.T. WANG, C.Y. WANG, H.F. CHEN. A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chem.*, 101, pp. 1753-1758 (2007).
12. Z. HOSEN. Light emitting diodes increase phenolics of buckwheat (*Fagopyrum esculentum*) sprouts. *J. Plant Interaction*, 2, pp. 71-78 (2007).
13. H.G. CHOI, B.Y. MOON, N.J. KANG. Effects of LED light on the production of strawberry during

- cultivation in a plastic greenhouse and in a growth chamber. *Sci. Hortic.*, 189, pp. 22–31 (2015).
14. X. HAO, J.M. ZHENG, C. LITTLE, S. KHOSLA. LED inter-lighting in year-round greenhouse mini-cucumber production. *Acta Hortic.*, 956, pp. 335–340 (2012).
 15. H.-M. LI, X.-M. LU, Q.-H. GAO. Effects of different light qualities on the growth, photosynthetic pigments and stomatal characteristics of okra (*Abelmoschus esculentus*) seedlings. *Acta Pratac. Sin.*, 25, pp. 26–70 (2016).
 16. E.M. BADEA, D. SĂNDULESCU. *Bio Tehnologii Vegetale*. Editura Fundația Biotech, București, 2001, pp. 21.
 17. D. CACHIȚĂ-COSMA, C. DELIU, L. RAKOSY-TICAN, A. ARDELEAN. *Tratat de biotehnologie vegetală*, Ed. Dacia, Cluj-Napoca, Vol. I., 2004, pp. 105.
 18. I.M. ENACHE, O. LIVADARIU. Preliminary results regarding the testing of treatments with light-emitting diode (LED) on the seed germination of *Artemisia dracunculus* L. *Scientific Bulletin. Series F. Biotechnologies*, ISSN 2285-1364, Vol. XX, pp. 52, 53 (2016).
 19. C. DONG, Y. FU, G. LIU, H. LIU. Low light intensity effects on the growth, photosynthetic characteristics, antioxidant capacity, yield and quality of wheat (*Triticum aestivum* L.) at different growth stages in BLSS. *Advances in Space Research*, 53: 11, pp. 1557 (2014). (<http://www.sciencedirect.com/science/article/pii/S0273117714000921>)
<https://doi.org/10.1016/j.asr.2014.02.004>
 20. M.M. BRADFORD. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254, (1976).
 21. V. MIHAILOVIĆ, S. MATIĆ, D. MIŠIĆ, S. SOLUJIĆ, S. STANIĆ, J. KATANIĆ, M. MLADENOVIĆ, N. STANKOVIĆ. Chemical composition, antioxidant and antigenotoxic activities of different fractions of *Gentiana asclepiadea* L. roots extract. *EXCLI Journal*, 12, pp. 807-823 (2013).
 22. K. MARXEN, K.H. VANSELOW, S. LIPPEMEIER, R. HINTZE, A. RUSER, U.-P. HANSEN. Determination of DPPH radical oxidation caused by methanolic extracts of some Microalgal Species by linear regression analysis of spectrophotometric measurements. *Sensors*, 7, pp. 2080-2095 (2007).
 23. J. ZHISHEN, T. MENGCHENG, W. JIANMING. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64, pp. 555-559 (1999).
 24. O. LIVADARIU, C. MAXIMILIAN. Studies regarding treatments of LED-s emitted light on sprouting *Fagopyrum esculentum* Moench., *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies*, 74: 2, pp. 102 – 111, (2017).