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# DETERMINATION OF ASSIMILATOR PIGMENT CONTENT IN CLADODES OF *OPUNTIA FRAGILIS* VAR. *FRAGILIS* EXPOSED TO LIGHT OF DIFFERENT COLORS EMITTED BY LEDs

**Teodora Iuliana VIDICAN[1], Dorina CACHITA-COSMA[2]**
[1] University of Oradea, Romania
[2] "Vasile Goldis" Western University of Arad, Romania

**ABSTRACT.** After determining the assimilator pigment content of *Opuntia fragilis* var. *fragilis* cladodes, from vitrocultures exposed for 90 days at a light intensity of 1000 lux, different colors (white, blue, yellow, red or green), issued by the LED (Light Emitting Diode), it has been found that in relation to their level, similar vitrocultures illuminated with white fluorescent light (control variant, the reference), only *Opuntia* vitroplantlets exposed to white light emitted by LEDs held chlorophyll a and carotenoid pigments in quantities close to the value determined from material plant derived from control samples. Instead, those vitroplantlets exposed to emitted red or yellow LEDs light, had a content of more than 50% below the lower chlorophyll a and chlorophyll b, in carotenoid pigments respectively, compared with that recorded in similar vitrocladodes from the culture illuminated with white fluorescent tubes (control variant). The strongest inhibitory effect on the assimilator pigment level gained from *Opuntia* cladodes mesophyll, regenerated in vitro, it has been challenged by green LED light, the vast majority of the examined pigments, the amount of which were approximately 30% from the parameters that have been registered with prepared extracts from control samples.

**Keywords:** *Opuntia*, LEDs, light, pigments, vitrocultures

## INTRODUCTION

It is well known the role and importance of the light in plants life. The intensity and nature of the wavelength, acts differently on photosynthesis, growth, transpiration and respiration of plants, regulates the opening and closing movements of ostiole stomata, conditioning the formation of chlorophyll, inhibit seed germination etc.

In the case of plant tissue culture, in general, photosynthesis is reduced; the required energy useful for life processes is obtained by sugar hydrolysis (usually sucrose) present in the medium culture. In such conditions, the light is stimulating vitroplantlets growth and influencing morphogenesis.

The spectral composition of sunlight consists of visible and invisible radiation. Visible radiation, namely: red, orange, yellow, contribute to synthesize carbon hydrants, while the blue favors particular protein synthesis, phototropism, inhibits hypocotyl elongation and opens the stomata in *Arabidopsis thaliana* (after Folta and Spalding, 2001), and stimulates photosynthesis in *Laminaria saccharina* (L.) (after Maier and Schmid, 1997), the green light promotes early growth in length to *Arabidopsis* stems (Folta, 2004), and to ferns, the white and red light delays the chlorophyll degradation and installation of senescence processes (Beherit and Biswal, 1990) etc.

Lighting LEDs *(Light Emitting Diode)* are small devices, which - depending on their mode of production - lights emit different colors: red, blue, yellow, white or green. LEDs are a creation of the electronic industry of the last decades of the twentieth century. Initially, they were used as optical devices, being inserted into various installations. The further development of LED's areas of application was fastly becoming expanded, because of the differences in spectra and intensity of light emission produced by them. Also, LEDs have a low residual emission, heat and UV radiation, and is characterized by low power consumption and least of all the LEDs have a less destructive impact on the environment. In figure 1 we illustrated the image of a LED (Fig. 1 A) mounted on its power cables with the voltage between 2 and 12 V, and a variant of the panel comprised of several LEDs assembled on a suitable support, shielded with a aluminum foil to reflect light emitted by them (Fig. 1 B).

Jao and Fang (2005) analyzed the effects on Solanum tuberosum vitrocultures illuminated by red light LED (wavelength (λ) of 645 nm), blue light (λ = 460 nm), by fluorescent red lamps (λ = 600 nm), blue light (with λ = 452 nm), these variants were used as reference data, i.e. as proof. Following this experiment, the authors concluded that the cultures that were lighted by the LED did not affect the vitroplantlets growth on the contrary the values for all growth parameters were higher than those marked on control variants the recorded average was increased with 35%. Following the efficiency calculations, made on the energy plan, it was found that vitrocultures with LED illumination were enabled by financial savings with 17% compared to the cost of fluorescent tube lighting with similar cultures.

***Correspondence:** Teodora Vidican, University of Oradea, Romania, E-mail: teodora.vidican@gmail.com*
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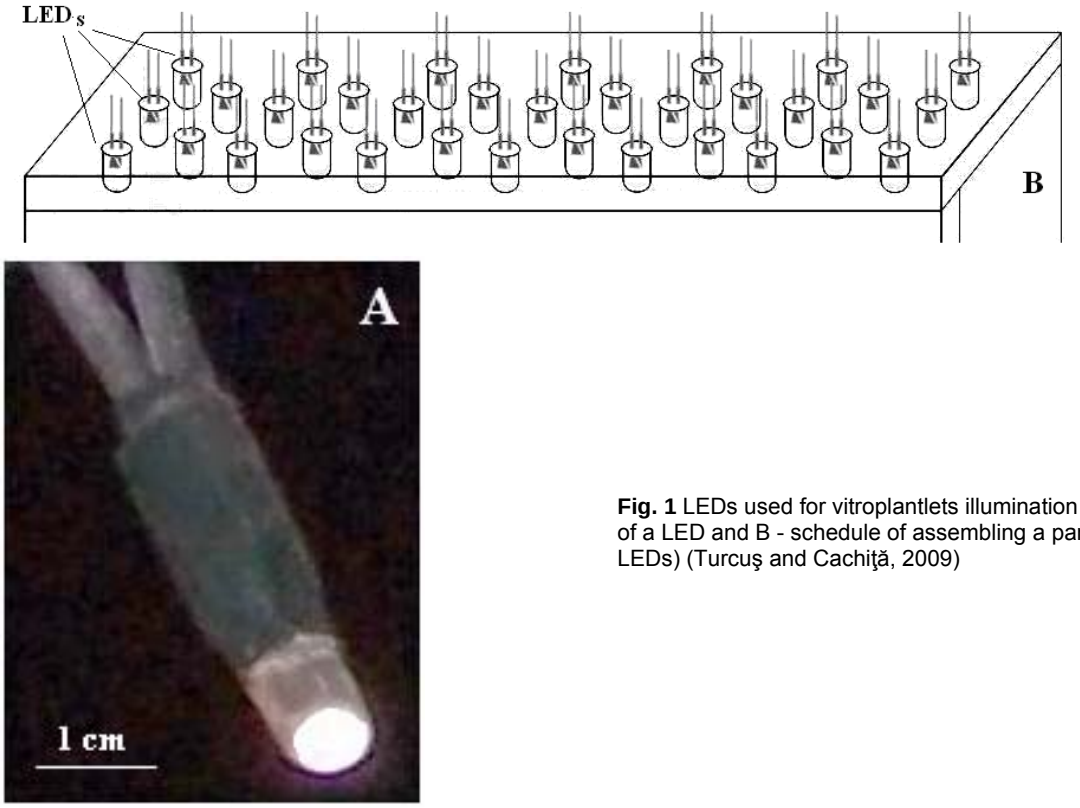


**Fig. 1** LEDs used for vitroplantlets illumination (A - image of a LED and B - schedule of assembling a panel with LEDs) (Turcuş and Cachiţă, 2009)

After Shemorakov (2001), an optimum illumination of cacti influences the nature and level of plastid pigments.

In Sequoia sempervirens vitroplantlets, Pop and Cachiţă (2007) showed that illumination with red LEDs on vitrocultures could be compared to similar cultures exposed to fluorescent light at the same color and intensity. Studies have shown that the assimilated pigment content by the plant material was increased.

The research that covers the present work, represents the continued new experiments with cactus vitrocultures (Vidican and Cachiţă, 2009), and refers to the studies of the changes in the assimilated pigment content of illuminated Opuntia vitroplantlets - for 90 days - with LED lights emitting various colors, compared to the determined level of these pigments in similar cultures illuminated by fluorescent white light.

## MATERIALS AND METHODS

We developed the initiation of Opuntia fragilis var. fragilis vitrocultures using caulinar explants, taken from young stems, harvested from mother plants grown in greenhouses (see Vidican and Cachiţă, 2010).

For this, the plant material was sterilized - by submersion - in 96º ethyl alcohol for one minute, after which it was covered with a 0.8% sodium hypochlorite solution compared to 1:2 mixed with sterile water, to which were added - as surfactant - three drops of Tween 20. In the presented environment, plant fragments were shaken continuously. After 20 minutes the disinfectant agent, has been removed and the plant material has been washed in sterile water in five consecutive rinsings, one at every five minutes. Next, the plant material was placed on aseptical filter paper washers that was placed in sterile Petri capsules.

In aseptic conditions in laminar flow hood perimeter of sterile air it has dimensioning explants sizes, which are shaped pieces of about 1cm long, 0.5 cm thick and 1 cm in diameter. Explants inoculation was made on a basal culture medium (BM), Murashige-Skoog (1962), Heller microelements (1953), to which were added vitamins: HCl pyridoxine, HCl thiamine and nicotinic acid (each 1 mg / l), meso-inositol - 100 mg / l, 30 g / l sucrose and 7 g / l agar – agar; the pH culture medium - through autoclaving - has been set to a value of 5.7. The culture media was devoid of growth regulators.

In the performed experiments varied the light source, especially the wavelength (λ) of radiation light emitted by it, namely:

V0 – illumination with white fluorescent tubes – control variant;

V1 – illumination with white emitted LEDs (λ = 510nm);

V2 – illumination with blue emitted LEDs (λ = 470nm).

V3 – illumination with yellow emitted LEDs (λ = 580nm);


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V4 – illumination with red emitted LEDs (λ = 670nm);

V5 – illumination with green emitted LEDs (λ = 540nm).

Phytoinocules containers were placed in boxes covered with lids that were installed on monochrome LEDs, placed in the growth room on shelves; the LEDs were am placed at a distance of 10 cm above vials with phytoinocules. The vial of control variant was placed in to the growth room, on shelves illuminated by white fluorescent tubes. In the whole experiment, the photoperiod was identical: 16 hours light from 24 hours; and the thermal light intensity was 1000 lux regime varied between 20-24°C. Vitrocultures were maintained under these conditions for a period of 90 days. In this time, on the explants level has regenerated to the cladodes, with an average size of approximately 4 cm (Fig. 2). After the vitroculture has reached the duration of the experiment it has been interrupted, and it was performed determination of the assimilating pigment taken in samples of Opuntia vitrocladodes, illuminatied applied during their in vitro growth process, with different kind of light.

## MATERIALS AND METHODS

The recommended methods of the Association of Official Analytical chemists [AOAC, 1999] were used for the determination of moisture, ash, crude lipid, crude fibre and nitrogen content.

***Sample collection and treatment.*** Plant samples were collected only after careful observation to determine areas where plants grow. Three samples were collected only during adulthood. Samples were labeled and it was noted aspect of the sampling. Dried vegetable products, is ground until a fine powder and then homogenized by sieving through a sieve with 30 mesh/cm$^2$. For a comprehensive chemical analysis of plant product was used in the original method of extraction of plant material with different polarity solvents. First – cut vegetable products (50.0 g) to a fine powder was extracted in Soxhlet apparatus with dichloromethane (non – polar solvent, the soluble lipophilic substances). After complete drying vegetable product remained was extracted with methanol (a solvent of medium polarity, which amphiphil soluble substances) and, finally, the residue was extracted with a highly polar solvent – water.

***Proximate analysis.*** The recommended methods of the Association of Official Analytical chemists were used for the determination of moisture, ash, crude lipid, crude fibers and nitrogen content.

***Mineral analysis.*** The mineral elements comprising sodium, calcium, potassium, magnesium, and phosphorus were determined with some modifications. 2.0 g of each of the processed samples was weighed and subjected to dry ashing in a well – cleaned porcelain crucible at 5500C in a muffle furnace. The resultant ash was dissolved in 5.0 ml of $HNO_3$/HCl/$H_2O$ (1:2:3) and heated gently on a hot plate until brown fumes disappeared. To the remaining material in each crucible, 5.0 ml of de – ionized water was added and heated until a colorless solution was obtained. The mineral solution in each crucible was transferred into a 100.0 ml volumetric flask by filtration through Whatman No. 42 filter paper and the volume was made to the mark with de – ionized water. This solution was used for elemental analysis by atomic absorption spectrophotometer. A 10 cm long cell was used and concentration of each element in the sample was calculated on percentage (%) of dry matter i.e. mg/100 g sample. Phosphorus content of the digest was determined colorimetrically.

***Vitamins analysis.*** Ascorbic acid (vitamin C) was determined titremetrically.

***Preparation of fat free sample.*** 2.0 g of each of the processed sample was defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 h.

***Other analysis.*** The moisture content of fresh parathas was determined. The parathas were then dried at 700 ± 20C in hot air oven, powdered and stored in glass bottle for further studies. The dried samples were then analyzed for true protein, crude fat, total sugar, mineral and energy by multiplication method. For biochemical characteristics, standard methods of analysis were used. The mineral content of samples was analyzed by AOAC method using the Atomic absorption spectrophotometer.

***Estimation of energy value.*** The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57 respectively) used in vegetable analysis.

The caloric value was determined based on the Atwater factor (FAO, 2006a).

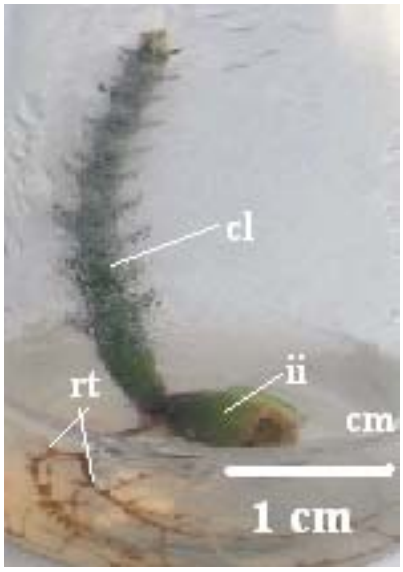


**Fig. 2** *Opuntia fragilis* var. *Fragilis* vitroplantlet, 90 days after explant in vitro culture, illuminated with white light fluorescent tubes, control variant (where: ii - the initial inoculum; cm - culture medium, cl - cladodes; rt - roots)


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Extraction of assimilator pigments it has been made separately for each experimental variant, with pure dimethylformamide (DMF, 99.9%). Working method was consisted by pressing of 50 mg cladodes fragments in 5 ml DMF (by Moran and Porath, 1980); the achieved composition was maintained for 72 hours at a temperature of 4ºC; then, supernatant was decanted, and the resulting solution content determinations were made for assimilated pigments by extract photometration from a Spectrophotometer SPEKOL 11 type. The operation itself, to determine the content of pigments in the liquid extract was made by photometration of samples using selective filters with different wavelengths, as follows: 664 nm for chlorophyll **a**, 647 nm for chlorophyll **b** and 480 nm for carotenoid pigments. For each experimental variant were made five repetitions.

The obtained data from photometration process were processed mathematically, as the formulas proposed by Moran and Porath (1980):

chlorophyll a (mg/gSP) = (11,65 $A_{664}$ – 2,69 $A_{647}$) * v/sp

chlorophyll b (mg/gSP) = (20,8 $A_{647}$ – 3,14 $A_{664}$ ) * v/sp

carotenoides (mg/gSP) = (1000 $A_{480}$ – 1,28 chlorophyll **a** – 56,7 chlorophyll **b**) / 245 * v/sp

where: $A_{480}$ – the readable value of 480nm filter;
$A_{647}$ – the readable value of 647nm filter;
$A_{664}$ – the readable value of 664nm filter;
v – ml used solution;
SP – mg fresh vegetal material used for extraction / sample; chlorophyll **a** and **b** – the quantity was calculated in mg for the first two formulas.

Averages of five repetitions were performed per experimental variant which was operated in the rest of the calculations.

By adding the average data obtained from determinations of chlorophyll **a**, with those from tests carried out to identify levels of chlorophyll **b** was obtained in total content of green pigment, by adding to these numbers the average values resulting from evaluation of carotenoid pigments content resulted from a complete picture of the level of determined assimilator pigments from *Opuntia fragilis* var. *fragilis* vitrocladodes, after 90 days of vitroculture exposed to various lighting regimes.

The results obtained in the case of extracts derived from lots of exposed *Opuntia* vitrocultures to light with LEDs have been reported to values of similar pigments samples belonging to control variant - vitrocultures illuminated with white fluorescent tubes ($V_0$) - data considered as benchmarks, as 100%. These data were either represented as histograms in figure 3.

## RESULTS AND DISCUSSIONS

As seen in figure 3, the highest values - in terms of total chlorophyll **a** content of extracts was present in vitrocultures illuminated by white LED light ($V_1$); this variant, compared to the control variant ($V_0$ - vitrocultures illuminated by white fluorescent tubes). Chlorophyll **a** content in extract marked a only 10.6% deficiency of and -17%, in the samples taken from batch cultures of *Opuntia* illuminated by blue LED light ($V_2$) (Fig. 3). The vitrocultures illuminated by red LED light ($V_4$) or yellow LED light ($V_3$) - this parameter has shown a decrease of 47% values representing the chlorophyll **a** content of samples, respectively 43% - compared to control variant ($V_0$) - at the cultures illuminatied with yellow LEDs. The vitrocultures illuminated by green LED light ($V_5$), the chlorophyll **a** content - compared to that recorded control variant ($V_0$) - was the lowest, the deficient recorded value being 63%.

In relation to variant $V_0$ (control variant), cultures illuminated by white fluorescent tubes (Fig. 3), the highest values of chlorophyll **b** content of total pigment extract made in DMF were recorded at all the samples prepared from vitrocultures of *Opuntia* illuminated by white LED light ($V_1$), but the concentration of chlorophyll **b**, at this sample was reduced with 31.3% compared with chlorophyll **b** for the control variant ($V_0$). The variant $V_5$, vitrocultures of *Opuntia* illuminated by green LED, the level of chlorophyll **b** in the extract was the lowest - the recorded deficiency value was 77% - from the control variant, and 74.7% at variant $V_4$ - vitrocultures illuminated by red LED light (Fig. 3). The same parameter was lower than that determined in control variant - with 61.2% - in the case of the variant $V_3$ - vitrocultures illuminated by yellow LED light and 60% in variant $V_2$ - vitrocultures illuminated by blue LED light. From the presented bellow resulting the fact that, generally, the illuminated of *Opuntia* vitrocultures with LEDs, had a negatively influence, particularly in chlorophyll **b** content in cladodes, especially after ist exposure to red or green light of the cultures. To note is that the *Opuntia* vitrocultures lighting with blue LED light decreased to 1/3 especially the concentration of chlorophyll **b** from cladodes.

Regarding the concentration of in the total of green pigments extracts prepared from plant material originating from different experimental variants from compared with control variant ($V_0$), reference values considered as 100%, to variants: $V_1$ - cultures illuminated with white LED light were this parameter marked a 20% deficiency; the variant $V_2$ - cultures illuminated with blue LED light, the values of green pigment content - in total extracts - was reduced with 36.7%, compared to control variant; the variant $V_3$ - cultures illuminated with yellow LEDs, the concentration of these pigments was ½ of the value of this parameter resulted in control variant, respectively with the total green pigment was reduced 60%, and in extracts pigments preparations from the vitrocultures illuminated with red or green light, emitted by LEDs, ahis values were reduced with 70%. Deducting from those shown before and that the light emitted by LEDs induced a decrease in total pigment chlorophylls


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content, especially in chlorophyll **b**, in the condition of *Opuntia* vitrocultures lighted with colored light; the descending order of the results were: blue, yellow and, in particular, red and green light (Fig. 3).

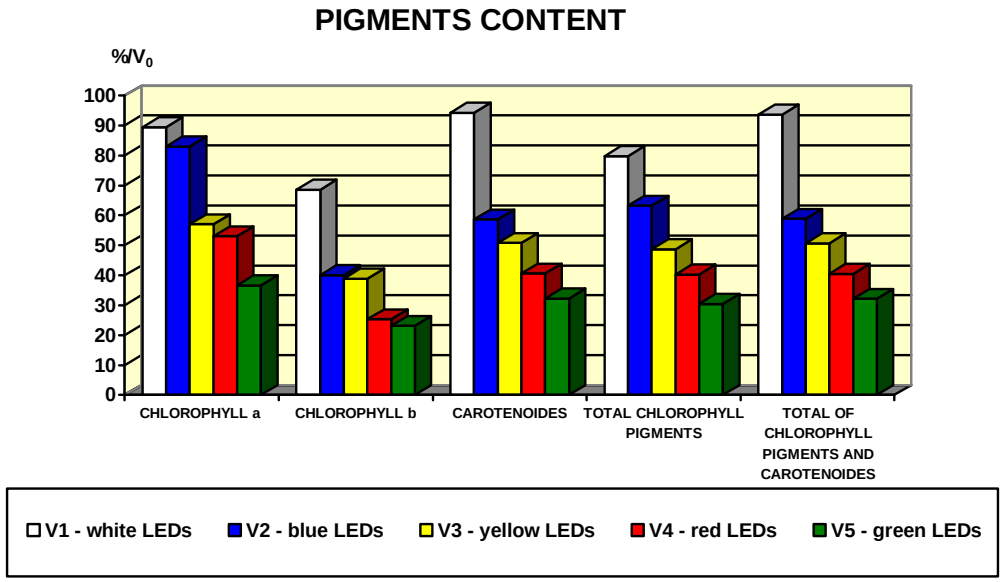


**Fig. 3** Assimilator pigment content in vitroplantlets of *Opuntia fragilis* var. *fragilis* 90 days after explants inoculation; the cultures were exposed to the light emitted by LEDs of different colors; experimental data were reported to the recorded values in the control variant ($V_0$), values considered as 100% (phytoinocules exposed to white fluorescent light)

Carotenoid pigments (Fig. 3) showed the higher values throughout the $V_1$ variant, cultures illuminated with white LEDs, marking a decrease of only 5.7%, in comparation with the level of the extract obtained from cladodes derived from vitrocultures exposed for 90 days to such light. In other experimental variants exposed to green light LED ($V_4$), the percentage of carotenoid pigments was decreased with 68% and in variant $V_5$ - vitrocultures exposed to blue light LED of these pigments level, in the extract prepared from *Opuntia* cladodes, it was decreased with 41%.

A decrease of 49% of the carotenoid pigment content was recorded in the prepared extracts obtained from regenerated cladodes at the vitrocultures from *Opuntia* illuminated by yellow LED light ($V_3$) and 59.5% in vitrocultures from *Opuntia* illuminated with red LEDs ($V_4$) in comparison with the similar pigments determined in cladodes from the lighting variant with white fluorescent light ($V_0$), values considered 100%.

From the histogram level analysis represented in figure 3, regarding to total assimilator pigment content resulted from the addition of data from the total sum of the carotenoid pigments with chlorophyll pigments, could be inferred that the light emitted by the white LED lights ($V_1$)resulted a decrease of only 6% of this parameter, while in the ca se of blue light ($V_2$) we registred a decrease of this parameter with 41.2%; in the case of yellow light LED illumination ($V_3$) determined a reduction of the with 49%, the red LED light ($V_4$) with 60% and the green LED light ($V_5$) with 68%.

## CONCLUSIONS

At 90 days after initiation *Opuntia fragilis* var. *fragilis* vitroculture it was found that, in exposed explants to white LED light, in the regenerated cladodes the level of chlorophyll **a** and **b**, respectively in the total chlorophyll pigments, but also in carotenoides pigment, compared with the similar parameter values registered to the samples from control variant (baseline data as 100%) – illuminated with white light emitted by fluorescent tubes, were decreased with 10.59% in the case of chlorophyll **a** and with 31.38% in the case of chlorophyll **b**, respectively only 5.72% in carotenoid pigments.

Blue light, emitted by LEDs determined a decrease of 17% of chlorophyll content in cladodes of *Opuntia*, regenerated at the explants vitrocultivated level, while the recorded values in chlorophyll **b** and carotenoid pigments have been registered a deficiency of 59,92%, respectively 41.1%, compared to the values of these parameters to the marked parameters at the variant with vitrocultures illuminated with white fluorescent lamps, reference data considered 100%.

Vitrocladodes of *Opuntia fragilis* var. *fragilis* exposed to red, yellow or green light, presented a reduced content of over 50% in chlorophyll pigments **a** and **b** (i.e. the total green pigment), but in the carotenoides, compared with similar values recorded in those parameters to control variant, cultures illuminated by fluorescent white light.


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Green LEDs, showed the strongest inhibitory effect both on the synthesis and content in chlorophyll and carotenoides pigments of *Opuntia* cladodes.

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