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EFFECT OF LED LIGHTING ON GROWTH AND PHENOLIC CONTENT ON *IN VITRO* SEEDLINGS OF *OCIMUM BASILICUM* L. CULTIVAR „*AROMAT DE BUZAU*"

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Abstract: *The aim of the experiment was to compare the effect of light emitting diodes - LED and fluorescent lamps (FL)d as sources of light on ,,in vitro" growth and development of sweet basil (Ocimum basilicum L. cultivar ,,Aromat de Buzau"). Sweet basil is an important medicinal and aromatic plant used in aromatherapy, cosmetics, perfume and food products in fresh or dried form. A major difficulty in the use of Lamiaceae species for pharmaceutical purposes is the individual variability, due to genetic and biochemical heterogeneity. In vitro, micropropagation is an effective mean for rapid multiplication of species in which it is necessary to obtain a high progeny uniformity. Therefore, the interest in using these techniques for rapid and large-scale propagation of medicinal and aromatic plants has been significantly increasing. Basil seedlings were grown under four monochromatic lights irradiated with blue, green, yellow, red LEDs with peak wavelengths of 470, 500, 525 and 660 nm.Basil plants produced greater fresh herbage mass as well as shoot height under fluorescent lamps used as control after 60 days of light treatment. However, the total leaf area of lateral shoots was the largest one under red light. The total phenolic content of basil plants was significantly higher under blue LED illumination as compared to the rest of the treatments. Also, LEDs increased the amount of flavonoids compared to FL light. Our study demonstrates that LEDs did not affect growth characteristics and increased the total phenolic content as compared to conventional fluorescent.*

Keywords: *Ocimum basilicum L. cultivar ,,Aromat de Buzau", light emitting diodes – LED, phenols, flavonoids, antioxidant capacity.*

1. Introduction

Plant development can be influenced by modulating light quantity and quality, as well as the photoperiod [1], the response of plants being recorde at chemical, morphological, and physiological level. Plant growth equipment (phytotrons) usually rely on conventional light emissions such as fluorescent tubes (especially cool-white) often used in combination with additional sodium and/or

incandescent sources, in order to obtain a complex light spectrum, closer to outdoors conditions [2]. Nevertheless, such light equipments present disadvantages, such as high energy requirement, short lifetime, and heat production. More recently, the use of light emitting diodes (LEDs) for plant cultivation in controlled conditions has appeared as an efficient, low-cost alternative technology [3]. LEDs are well suited for plant growth equipment, having reduced weight andvolume and long lifespan (approximately 100,000 hours). Moreover, LED lighting achieves major energy consumption reduction due to emission of very narrow wavelengths. In two varieties of lettuce [4], the results showed that blue LEDs are more efficient for bioactive substances production. For both varieties, increases of vitamin C and carbohydrates amounts were obtained using LED with 40% blue light and 60% red light. Furthermore, combining red and blue LEDs proved effective for photosynthesis considering the higher chlorophyll content in plants grown with LEDs light compared with plants grown with Ne source.

Research indicate that both light quantity and quality influence plant morphology. Regarding radishes, soybean and wheat grown with LEDs with different percentages of blue light, the results showed that blue light did not influence total dry weight, blue light from warm white LEDs led to augmented stem elongation and leaf growth, while blue light from cool white LEDs led to more compact plants. In the present, a viable option to reducing costs for commercial micropropagation is represented by replacements for cultivation mediums needed for commencing or subcultivation of vitroplantlets.

Sweet basil (*Ocimum basilicum* L.) is a India native specie [5] and is also encountered in tropical and temperate regions of Asia. The genus *Ocimum* is distributed in Asia, Africa and Central America [6] and is grown in several European countries such as Italy, France and Spain. In Turkey, basil is grown in Eastern Anatolian area, used as a medicinal and aromatic plant. Sweet basil presents large intrapsecific variations on a morphological level [7, 8] and also regarding the composition of essential oil [9] as well as other bioactive substances [10]. Basil is a highly appreciated medicinal and aromatic specie mainly as a result of the high content in essential oils obtained from leaves and flowers [11], containing phenolics [12] and of the presence of other non-volatile compounds such as flavonoids and anthocyanins [13].

In vitro propagation of medicinal species using tissue culture techniques provides an effective way of optimized production of secondary metabolites from in vitro induced callus or whole plants.

Although an important culinary and medicinal plant, few studies have been conducted on basil growth using LEDs as a light source.

In the same time, there is no extensive information regarding the relationship between light captured by basil and physiological mechanisms underlying the secondary metabolism production under the influence of different light quality. Secondary metabolites are produced to allow plants to overcome stressful conditions. In tissues such as stems and leaves, secondary metabolite synthesis can be influenced by environmental, physiological, biochemical and genetic factors [14, 15], light having a major influence [16]. One of the main groups of secondary metabolites is represented by phenolics. This group is one of the most ubiquitous class of secondary compounds

in plants and indicates high metabolic plasticity, enabling plants to adapt to biotic and abiotic environmental influences [14]. Their concentration varies with the season and with the stage of growth [14].

Phenolics are substances exhibiting free radical scavenging capacity, as well as inhibitory activity against microorganisms and insects [17]. Physiological changes are triggered by exposure to varying light quality [18]. Red light is the wavelength affecting biomass production and organ elongation through the phytochrome photoreceptor [19]. Blue light also influences photomorphogenic responses (such as leaf flattening and compact appearance) through phototropins and cryptochromes acting independently and/or synergically with phytochromes [20].

The present paper hypothesizes that pre-cultivating basil seedlings under light with varying wavelengths would differently influence morphological and chemical properties. In addition, different light emitting diodes spectra would induce varied morphological and phytochemical reactions among different basil cultivars. The objective of this research was (1) to investigate the effect of LED sources on morpho-chemical characteristics of ,,Aromat de Buzau" basil cultivar in vitro and (2) to determine the optimum wavelength for basil growth.

2. Materials and methods

2.1. Plant material, growth conditions and light treatments

The culture substrate used in the *in vitro* cultures experiments consisted of basal medium (MB) Murashige - Skoog (1962) (MS) agar medium, which consisted of macronutrients, Fe EDTA and trace elements, mineral mix according to the original recipe, but with increased addition of vitamins: pyridoxine HCl, nicotinic acid and thiamine HCl (1 ml / l each of the original recipe to where indicated 0,5 mg / l), to which was added m-inositol 100 mg / l, sucrose 30 g / l and agar - agar 10 g / l; this basic medium (MB) were added as growth regulators 0,5 mg / l IBA (indolyl butyric acid) and 0,5 mg / l BA (benzyladenine). Before autoclaving of the culture medium, the pH value was adjusted to 5,5 with HCl or NaOH, depending on the basicity or acidity of the final medium. For autoclaving, 15 ml of the medium were placed into the clear glass culture containers that were temperature resistant, 8 cm height and 4 cm diameter. After portioning the culture medium, the culture containers were filled with aluminium foil. Sterilization of the containers and culture media was performed by autoclaving at 121°C for 21 minutes.

The plant material used for the initiation of vitro cultures was the meristematic apexesof basil plants ,,Aromat de Buzau" cultivar with a length of about 1 cm and 2-3 leaf primordia, harvested from seedlings regenerated from zygotic embryos that have sprouted from 30 days old seeds that were germinated on a septic substrate consisting of peat mixed with perlite. After cooling of the culture medium, was performed the inoculation of explants on culture medium and then the inoculated containers were transferred into growth chamber that were placed on racks exposed to a temperature ranged from 23°C ± 2°C, in the light regime, 20°C ± 2°C, during darkness and a photoperiod of 16 h light / 24h. Fluorescent tubes emitting coloured light (Osram company) were used as a control, length 590 mm, Ø 26 mm, 120 lux light intensity and LEDs emitting colored light with different wavelengths: blue (465 nm), yellow (590 nm) and red (650 nm) from Osram company.

2.2. Quantification of phenols, flavonoids, antioxidant capacity

Extracts were prepared by macerating 5 g of ground fresh plant in 95 ml of distilled water or 30% w/v ethanol for 24 hours. Total phenolic content was assessed using the Folin Ciocalteu reagent method, by spectrophotometric readings at 760 nm of the colour of incubated extracts [26].
Total flavonoid content was determined according to the method described in Makri in 2008, by evaluating the absorbance at 510 nm of extracts reacted with 5% $NaNO_2$ and 10% $AlCl_3$.
The free radical scavenging capacity was assessed through the DPPH method where the relatively stable free radical can be reduced by electron-rich radical scavengers from medicinal plant extracts[27], measuring the decolouration of DPPH solution reacted with extracts at 515 nm for 3 hours.

2.3. Statistical analysis

The statistical analyses conducted were represented by analyses of variance among treatments and the Tukey test at $p<0.05$, the results being expressed as means and standard errors.

3. Results and discussions

3.1. Morphological and developmental measurements

Number of leaves, shoot height, root length, measurements were conducted during the two months experimental period (60 days). Specifically, shoot height was measured on a weekly basis with the first measure taking place 7 days after inoculation. After 60 days experimental period 10 randomly selected seedlings were sampled. Morphological characteristics such as leaf number, shoot height, root length were measured. At day 60, leaf number was not affected by the different light treatments, with FL (control) and LEDs forming around 4 and 5 leaves respectively (table 1). Shoot height was variably affected by the different light treatments. Seedlings grown under red light of LEds exhibited significantly higher shoots compared to control and others LEDs treatments.

Table 1.

Morphological indices of *Ocimumbasilicum* L. grown at different LED colors after 60 days.

Indices	Control	Blue light	Red light	Yellow light	Green light
Stem height (cm)	$5.92^{b}\pm0.05$	$4.08^{a}\pm0.08$	$6.06^{b}\pm0.07$	$5.08^{b}\pm0.09$	$5.82^{b}\pm0.06$
Root length (cm)	$6.94^{b}\pm0.06$	$5.1^{a}\pm0.02$	$7.2^{b}\pm0.08$	$5.4^{a}\pm0.04$	$7.12^{b}\pm0.06$
Number of leaves	$4.6^{b}\pm0.21$	$5.4^{b}\pm0.21$	$4.2^{b}\pm0.17$	$3.4^{a}\pm0.21$	$3.6^{b}\pm0.21$

(Average of 5 measurements ± standard error, different letters between rows represent significant statistical differences).

Regarding the root length, significant differences were found between red light that exhibited the highest average value and blue light treatment (figure 1 and figure 3). After 60 days of vitroculture, if comparing vitroseedlings in terms of growth, we can see that the red light and yellow stimulates increase in length of vitroseedlings and blue light produce small and stocky and even slowing growth of plants (figure 2).

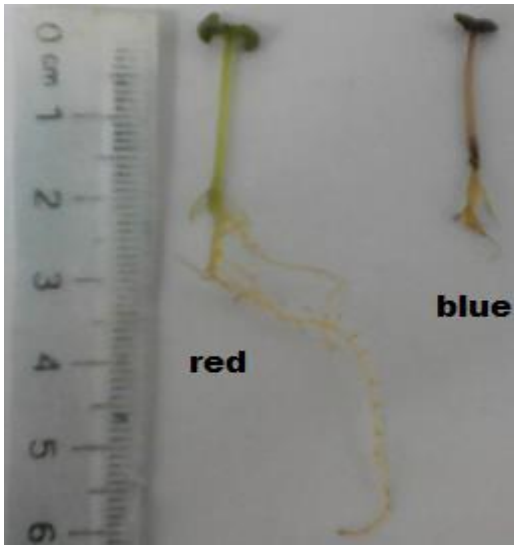

Fig. 1. Morphological aspects of *in vitro* seedlings basil after 30 days of cultureunder red lighttreatment and blue light treatment.

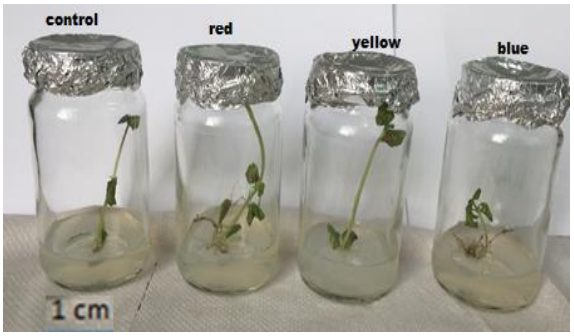

Fig. 2. Morphological aspects of *in vitro* seedlings basil after 60 days of cultureunder white fluorescent tubes (control) and light emitting diodes treatments(red, yellow, blue).

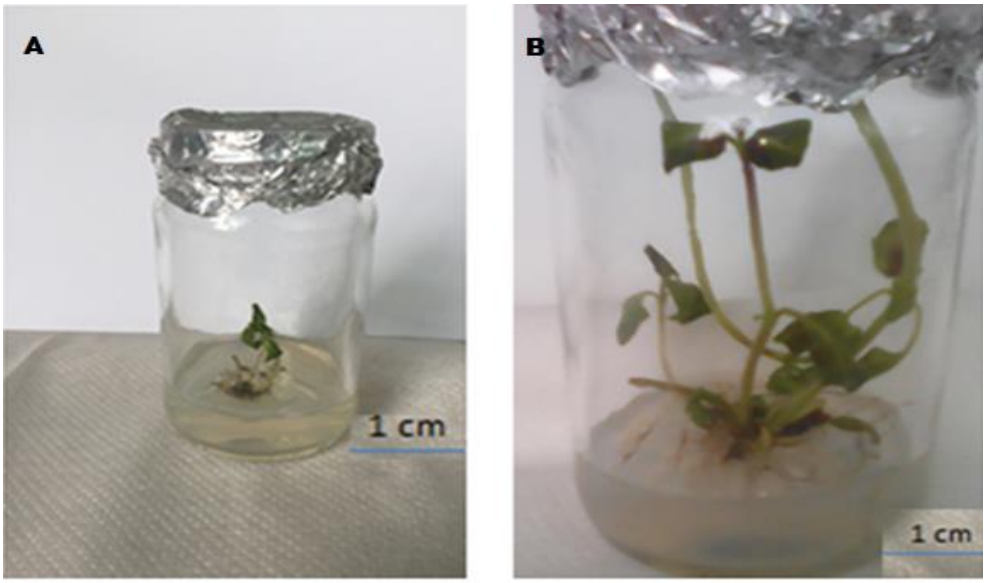

Fig. 3. Morphological responses of *in vitro* seedlings basil after 60 days of cultureunder blue lighttreatment (A) and red light treatment(B).

3.2. Phytochemical characteristics

The research on the basil in vitro plants, cultivated on Murashige-Skoog medium culture exposed 30 days in light fluorescent tubes and LEDs revealed that the lighting regimestimulated antioxidant compounds production. As can be seen in Figure 6, blue light treatment determines the highest amount of polyphenols, followed by the quantity produced in vitro plants grown under yellow light. Regarding assimilatory pigment, vitroseedlings grown in colored light

produced by LEDs contained the highest amount of assimilating pigments, especially those illuminated with blue light, followed by those illuminated with yellow and red light.

High yield using red light treatment is reported [22], and described in other species such as *Lycopersicum esculentum* [23]. The amount of carotenoids is related to green and blue light (0.4 to 0.48 mg/g), these pigments absorbing mainly at these wavelengths [24].

In this study, the antioxidative proprieties of basil were measured spectrophotometrically by DPPH assay. This method is preferred due to its simplicity, convenience and time-saving properties [25]. The extract, functioning as an antioxidant, reacts with DPPH (1-diphenyl-2-picrylhydrazyl), resulting in the formation of 1-diphenyl-2-picrylhydrazine.

Almost all variants of vitroseedlings (two months old leaf and stem) showed considerable antioxidant activity.

Nevertheless, among all the extracts assessed, the extract with the highest antioxidant activity was that of the leaf and stems illuminated with red light, (22,36% higher) than that of vitroplants illuminated with others colours of light (figure 6), especialy with blue light (14,86%).

Antioxidant capacity in plants is known to be variable, according to specie but also to the origin of the samples within the same specie [28]. However, considering the potential uses of antioxidant extracts in food applications [29,30], production of raw material with enhanced free radical scavenging activity is highly desirable.

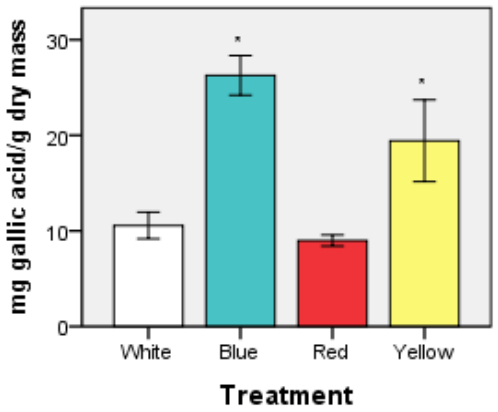

Fig. 4. Total phenolic content of basil extracts "Aromat de Buzau" cultivated under different color led lights. The mean difference is significant at the 0,05 level compared to control.

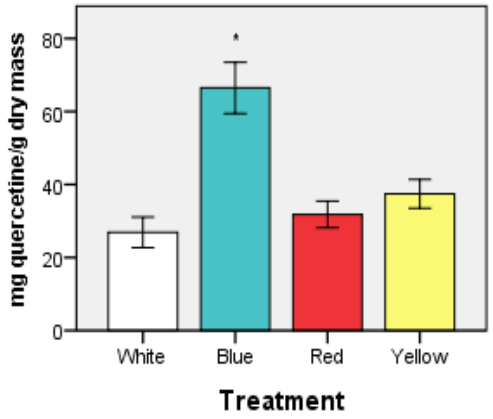

Fig. 5. Total flavonoid content of basil extracts "Aromat de Buzau" cultivated under different color led lights. The mean difference is significant at the 0,05 level compared to control.

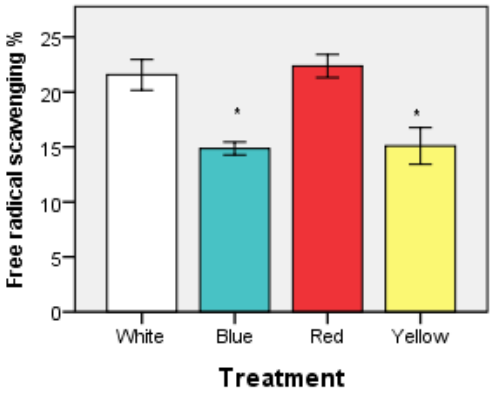

Fig. 6. Free radical scavenging capacity of basil extracts "Aromat de Buzau" cultivated under different color led lights. The mean difference is significant at the 0,05 level compared to control.

4. Conclusions

The results from the present study clearly suggested that basilplants may serve as an excellent source of antioxidants.
These findings are also beneficial in providing a useful benchmark in determining the optimum colour of light from this species at its best, most promising antioxidative effects.
The results of this study would be used to give guidance on light colour sources design for basil cultivation in a controlled environment.
In addition, it may serve as a basis for even more extensive researches to be done on this species with the focus of interest directed towards its phytomedicinal values, hence would be incorporated into health-promoting supplementary foods and pharmaceutical preparations.

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