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## The use of UV-A radiation for biofortification of lettuce and basil plants with antioxidant phenolic and flavonoid compounds

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#### ABSTRACT

Stable plant production is a crucial concern of modern agriculture facing increasing food demands and the risk of less predictable weather conditions in the open field. Alternative approaches for plant production are greenhouses and indoor farming. Modern LED-based artificial lighting indoor facilities allow not only to fill the gap in the supply chain of food production, but to produce plants characterized with higher concentration of essential phytochemicals. Thus, in this study, we attempt to analyse the efficiency of short-term supplementation of spectrum within ultraviolet A light (UV-A, 365 nm) to increase the antioxidant potential of leafy plants, assessed by total phenolic (TPC) and flavonoid (TFC) content. To this end, two distinct cultivars of baby leaf lettuce (Lactuca sativa var. crispa L.) and microgreens basil (Ocimum basilicum L.) plants were grown under redgreen-blue spectrum supplemented prior to harvest with low doses of UV-A radiation. Analyses showed that UV-A exposition enhanced TPC and TFC with green leaf cultivars, compared to dark-pigmented ones. The analysis also proved that plants exposed to UV-A presented higher overall antioxidant potential measured with 1,1-diphenyl-2-picrylhydrazil (DPPH). Results are crucial for better understanding the potential of UV-A supplementation to produce functional plants which are natural sources of dietary antioxidants.

**KEYWORDS:** indoor farming, spectrum optimization, baby leaf, microgreens, antioxidant potential

#### Introduction

The sustainability of resources and safety in the plant food production is a major issue globally. It is expected that by 2050, the global population will reach 9.8 billion people. Thus, it will force the necessity to enhance the total area of crop production (Avgoustaki and Xydis, 2020). At the same time, however, the

availability of land area for agriculture is projected to be restricted due to purposes such as urbanization, energy production, infrastructure, and predicted effects of global temperature rise, causing sea level increase (Despommier, 2011). Fortunately, indoor farming (IF)approaches have recently been identified as a potential alternative to contribute to sustainable plant food production (Specht et al., 2014). IF also provides the opportunity to create desired conditions for crop growth such as temperature, relative humidity, carbon dioxide level, air circulation, and artificial lighting quantity and quality, and at the same time mitigates open field disadvantages such as weather extremes or pathogens and pests (Ampim et al., 2022). Moreover, for most plant production lighting systems with non-saturating light intensity are sufficient (Trojak and Skowron, 2021), thus the light quality rather than quantity is more crucial during plant production.

Different light wavelengths have a significant impact on the nutritional content of food crops. Both visible and ultraviolet (UV) radiation can notably affect the secondary metabolism leading to the accumulation of health-promoting phytochemicals vital for human health (Lee et al., 2022). UV region (100-400 nm) is divided into three sub-regions, UV-A (315-400 nm), UV-B (280-315 nm) and UV-C (100-280 nm) (Darré et al., 2022). Plants cultivated in open-field conditions are naturally exposed to sunlight, which contains UV radiation (Surjadinata et al., 2017). As a result, plants evolved various metabolic and biochemical responses to UV exposition, among them increased antioxidant secondary metabolites synthesis (Brazaityte et al., 2015).

The underlying mechanism of plant UV responses is related to different photoreceptors – blue light/UV-A ones such as cryptochromes (crys) or UV

(UVR8) Resistance Locus 8 photoreceptors, which operates through UV-B light (D'Amico-Damião and Carvalho 2018). Perception and response to UV-C light are associated with the redox state of cells and reactive oxygen generation species (ROS) (Artés-Hernández et al., 2022). The master regulator of UV responses is the elongated hypocotyl 5 (HY5) transcription factor. Its UV-depended accumulation induces phenolic compounds biosynthesis, with largest class of flavonoids (Vanhaelewyn et al., 2020; Xiao et al., 2022). Consequently, UV radiation is considered as a tool to biofortify IF-grown crops with nutraceuticals (Jacobo-Velázquez et al., 2022).

Up to date, however, data on the UV-A effect on the accumulation of phenolic compounds is scarce and incomplete (Verdaguer et al., 2017). Thus, the aim of the study was to examine the efficiency of short-term supplementation of spectrum within UV-A (365 nm) in increasing the antioxidant potential of leafy plants, assessed by total phenolics (TPC) and flavonoids (TFC) content. To this end, baby leaf lettuce (Lactuca sativa var. crispa L.) cultivars with green (cv. Lollo Bionda) and red leaf (cv. Lollo Rossa), as well as microgreens basil (Ocimum basilicum L.) cultivars with green (cv. Sweet Large) and purple leaf (cv. Dark Opal), were grown in a growth chamber under red-green-blue (RGB) spectrum, supplemented prior to harvest with and increasing doses of UV-A. Spectrophotometric analyses clearly showed that UV-A exposition enhanced TPC and TFC as well as total antioxidant capacity (TAC) in both green-leaf cultivars, while both dark-pigmented cultivars presented slightly lower TPC and TFC content, compared to control plants. The study provides valuable insight into the role of UV-A supplementation in standard RGB lighting

UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS

systems, mostly devoid of UV components, to improve the quality of plant leafy products.

## Methods

#### Plant material and growth conditions

Baby leaf lettuce (L. sativa var. crispa L.) cultivars with green (cv. Lollo Bionda, LB) and red leaf (cv. Lollo Rossa, LR), as well as *microgreens* basil (O. basilicum L.) cultivars with green (cv. Sweet Large, SL) and purple leaf (cv. Dark Opal, DO), seeds were sown in P9 containers ( $9 \times 9 \times 10$  cm) and filled with the substrate (white and black peat, perlite, and N:P:K = 9:5:10; pH 6.0–6.5), divided into groups, and transferred to environmentally controlled growth chambers, with non-reflective black separators to eliminate light contamination. The plants were grown for the next 20 consecutive days (20 DAS, days after sowing) (Fig. 1) under LED RhenacM12 lamps (PXM,



**Figure 1.** Morphology of 20-DAS plants of baby leaf lettuce (*Lactuca sativa* var. *crispa* L.) cultivars with green (cv. Lollo Bionda, LB) and red leaf (cv. Lollo Rossa, LR) as well as *microgreens* basil (*Ocimum basilicum* L.) cultivars with green (cv. Sweet Large, SL) and purple leaf (cv. Dark Opal, DO) grown under RGB (C, control) or RGB+UV-A (UV-A supplemented) spectrum.



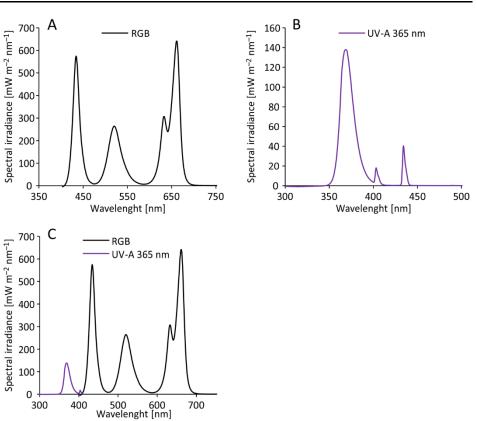
**Figure 1 (continued).** Morphology of 20-DAS plants of baby leaf lettuce (*Lactuca sativa* var. *crispa* L.) cultivars with green (cv. Lollo Bionda, LB) and red leaf (cv. Lollo Rossa, LR) as well as *microgreens* basil (*Ocimum basilicum* L.) cultivars with green (cv. Sweet Large, SL) and purple leaf (cv. Dark Opal, DO) grown under RGB (C, control) or RGB+UV-A (UV-A supplemented) spectrum

Podleze, Poland) delivering 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of the RGB spectrum (R:G:B; 661:633:520:434 nm) (Fig. 2A) or RGB+UV-A supplemented for 4 days prior to harvest with and gradually increasing daily doses – 60, 120, 240 and 480 min, respectively of UV-A lamp with peak wavelength 365 nm (9 W m<sup>-2</sup>) (Figs. 2B,C) (Actinic BL PL-S 9W/10/2P, Philips lighting, Eindhoven, The Netherlands). RGB treatment was used as the control group. Light composition and

photosynthetic photon flux density (PPFD) were monitored by a calibrated spectroradiometer GL SPECTIS 5.0 Touch (GL Optic Lichtmesstechnik GmbH, Weilheim/Teck, Germany).

The containers with plants were turned in twice a day. The photoperiod was 16/8 h (day/night; day 6:00 a.m.–10:00 p.m.), the average air temperature was maintained at 23/20 °C (day/night), relative air humidity was kept at 50–55% and 420  $\pm$  10 µmol mol<sup>-1</sup> of CO<sub>2</sub>. The

UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS



**Figure 2.** The light spectra of growth chambers were recorded with a spectroradiometer at six locations and then averaged. Plants in the RGB (red–green–blue) chamber were grown under 200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of the RGB spectrum (R:G:B; 661:633:520:434 nm) (A) or RGB supplemented with UV-A (B, C) (365 nm, 9 W m<sup>-2</sup>) for 4 days prior to harvest with increasing doses. RGB states for the control plants (R:G:B = 1:1:1).

plants were watered with tap water when necessary and fertilized once a week with 1% (w/v) fertilizer (N:P:K = 9:9:27; Substral Scotts, Warszawa, Poland). The top leaves from the plants 20 DAT were used for subsequent analyses. All analyses were conducted between 8:00 a.m. and 12:00 p.m. Ten plants (two repetitions with five plants per light condition) were grown with each kind of light treatment.

# Estimation of total phenolic content with Folin-Ciocalteu assay

Estimation of total phenolic content (TPC) was conducted, as described by Ainsworth and Gillespie (2007). In brief, 100 mg of fresh weight (FW) leaf tissue

(four replicates per light treatment) was placed in tubes with 1.0 ml of methanol. Samples, kept in dim light, were vortexed for 20 s and incubated for 30 min at 60  $^{\circ}\mathrm{C}$ with inversion every 10 min to improve extraction. Then, the sample mixture was centrifuged at  $10,000 \times g$  for 2 min, and then the supernatant was carefully collected without disturbing the plant tissue, transferred to a new tube, and mixed once again for 15 s. Then 100 µl of each extract, cooled down to room temperature (RT), was mixed with 200 µl 10% (v/v) Folin-Ciocalteu reagent (F-C) and vortexed twice for 10 s. Then 800 µl of 700 mM Na<sub>2</sub>CO<sub>3</sub> was added, vortexed twice for 10 s, and incubated for 30 min at 40 °C, protected from light. After incubation mixture was centrifuged at  $10,000 \times g$  for 1 min and transferred to a clear 96-well microplate with 200 µl per well. For TPC determination the absorbance at 765 nm was estimated with a microplate spectrophotometer (Mobi, MicroDigital Co., Ltd., Republic of Korea) with four replicates. The standard curve with gallic acid (0–200 nmol) was used to estimate nanomoles of phenolic compounds (gallic acid equivalents) in a sample.

#### Estimation of total flavonoid content

For the measurement of total flavonoid (TFC) assay proposed by Shraim et al. (2021) with modification was applied. The 60 µl of methanol extract obtained previously for TPC assay was mixed with 680  $\mu$ l of 30% (v/v) methanol: water and 30 µl of 0.5M NaNO<sub>2</sub>, vortexed for 20 s and incubated at RT for 3 min without light. Then 30 µl of 0.3M AlCl<sub>3</sub> x 6H<sub>2</sub>O was added to each sample vortexed for 20 s and incubated at RT for 3 min, and then mixed with 200 µl of 1M NaOH, vortexed and left for the next 40 min at RT without light. After incubation, samples were mixed, shortly centrifugated  $(5,000 \times g)$ for 1 min) and 200 µl aliquot of each sample were transferred to 96-well microplate. For TFC determination the absorbance at 506 nm was estimated with a microplate spectrophotometer with four replicates. The flavonoid content in the sample extracts was quantified using calibration curves of flavonoid standards of rutin

## Antioxidant activity by DPPH assay

The antioxidant activity of each extract of tested plants was measured by the 1,1-diphenyl-2-picrylhydrazil (DPPH) scavenging assay according to the method proposed by Mehmood *et al.* (2022). For DPPH assay the 60  $\mu$ l of plant methanol extract obtained previously for TPC assay was mixed with 904  $\mu$ l of methanol and 576  $\mu$ l of 0.125mM DPPH in methanol, vortexed for 20 s and incubated for 30 min at 37 °C. Using a microplate spectrophotometer, the absorbance of each sample was measured at 517 nm with four replicates. To determine sample radical scavenging activity, the calibration curve with a synthetic antioxidant – butylated hydroxytoluene (BHT) (0–400  $\mu$ g per ml) and 0.125mM DPPH was plotted.

The following formula was used to calculate the percentage of DPPH scavenging activity:

$$\frac{\text{DPPH}}{\text{inhibition }\%} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\%$$

- control states for DPPH mixture incubate with 0  $\mu$ g BHT solution.

#### Models for fitting and statistical analysis

The fitting of experimental data of DPPH inhibition by BHT used for DPPH radical scavenging activity rate was performed using OriginPro version 2024 (OriginLab Corporation, Northampton, MA, USA).

Statistical analyses were performed using Statistica 13.3 software (StatSoft Inc., Oklahoma, OK, USA). The normal distribution of variables was verified using the Shapiro–Wilk test, and the equality of variances was evaluated using Levene's test. One-way ANOVA and post hoc Tukey's HSD tests were employed to analyse the differences between the investigated groups. The data are presented as mean with standard deviation ( $\pm$ SD). Statistical significance was determined at the 0.05 level (p = 0.05).

#### Results

#### *Total phenolic content*

Estimated total phenolic content (TPC) is expressed as nmol gallic acid equivalents per mg of fresh weight (FW) (Fig. 3). Analysis showed that dark-

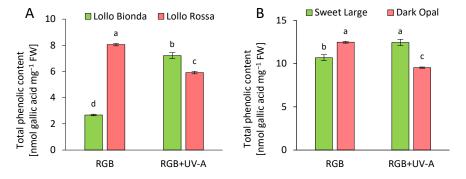
UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS

pigmented cultivars of both species presented higher phenolics content under the control RGB spectrum. In lettuce leaves TPC is almost 3 times higher for LR than LB cultivar (Fig. 3A), while for basil the difference is about 17% higher for DO than SL (Fig. 3B). Yet, basil leaves have significantly higher TPC than lettuce ones. The short-term exposition of leaves to additional UV-A light increased TPC significantly in green-leaf cultivars, as we observed 170% and 16% higher TPC for LB and SL, respectively. In contrast, UV-A decreased TPC by 27% and 24% in LR and DO, respectively.

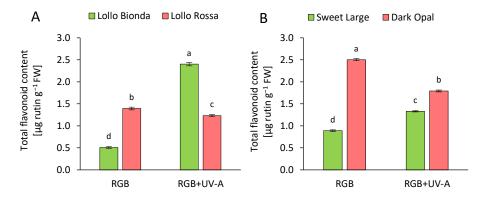
#### Total flavonoid content

Total flavonoid content (TFC) is expressed as  $\mu$ g rutin equivalents per g of fresh weight (FW) (Fig. 4).

Similarly to TPC, also flavonoids content was significantly higher in darkpigmented cultivars. Under RGB spectrum, we documented 2.7 times higher TFC level in the LR compared to



**Figure 3.** Total phenolic content (TPC) of control (RGB) or UV-A treated (RGB+UV-A) plants of *baby leaf* lettuce (*Lactuca sativa* var. *crispa* L.) cultivars with green (cv. Lollo Bionda) and red leaf (cv. Lollo Rossa) (A) or *microgreens* basil (*Ocimum basilicum* L.) cultivars with green (cv. Sweet Large) and purple leaf (cv. Dark Opal) (B) 20 DAS, estimated as nmol gallic acid equivalents per mg of fresh weight (FW). Each bar represents the average  $\pm$  SD of four independent measurements (n = 4). Different letters (a–d) indicate significant differences between treatments at p = 0.05 with a Tukey's HSD test.



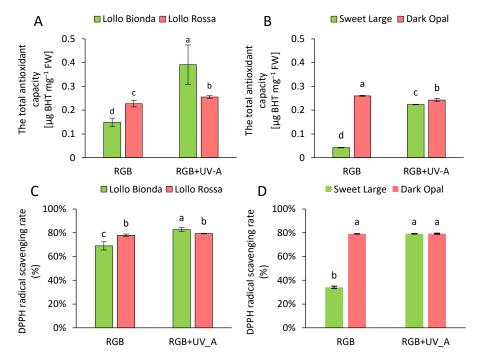
**Figure 4.** Total flavonoid content (TFC) of control (RGB) or UV-A treated (RGB+UV-A) plants of *baby leaf* lettuce (*Lactuca sativa* var. *crispa* L.) cultivars with green (cv. Lollo Bionda) and red leaf (cv. Lollo Rossa) (A) or *microgreens* basil (*Ocimum basilicum* L.) cultivars with green (cv. Sweet Large) and purple leaf (cv. Dark Opal) (B) 20 DAS, estimated as  $\mu$ g rutin equivalents per g of fresh weight (FW). Each bar represents the average  $\pm$  SD of four independent measurements (n = 4). Different letters (a–d) indicate significant differences between treatments at p = 0.05 with a Tukey's HSD test.

the LB cultivar, and approximately 2.8 times higher in DO than in the SL basil cultivar. Supplementation of RGB spectrum within UV-A light increased flavonoids content by almost 4.7- and 1.5-fold in green-leaf cultivars of lettuce (Fig. 4A) and basil (Fig. 4B), respectively. UV-A also showed negative impact noticed with 12% and 28% TFC decrease in LR and DO, respectively.

#### Overall antioxidant capacity

The total antioxidant capacity (TAC) was expressed as  $\mu g$  equivalents of butylated hydroxytoluene (BHT) per mg of FW (Figs. 5A,B) and DPPH radical scavenging activity rate (Figs. 5C,D) was assessed based on fitted experimental data of BHT calibration curve, as described in Methods. As expected, analysis proved

that overall antioxidant capacity complies with TPC and TFC. Under spectrum depleted in UV-A radiation green leaf cultivars showed 1.5- and 6.5-times lower antioxidant capacity compared to darkpigmented ones in lettuce and basil plants, respectively. Despite short-term exposition of plants within UV-A light, it was efficient in antioxidant capacity increase. In the LB lettuce TAC was increased by 162% (Fig. 5A), while in the SL basil by 460% compared to RGBgrown plants (Fig. 5B). In the case of LR cultivar presenting reddish leaf phenotype UV-A light treatment increased TAC by 12%. On the other hand, UV-A lowered TAC by 7% in DO basil. Estimated DPPH radical scavenging activity rate shows that UV-A exposition increased its value by approximately 14% and 45% in LB and



**Figure 5.** The total antioxidant capacity (A, B) and DPPH radical scavenging activity rate (C, D) of control (RGB) or UV-A treated (RGB+UV-A) plants of *baby leaf* lettuce (*Lactuca sativa* var. *crispa* L.) cultivars with green (cv. Lollo Bionda) and red leaf (cv. Lollo Rossa) or microgreens basil (*Ocimum basilicum* L.) cultivars with green (cv. Sweet Large) and purple leaf (cv. Dark Opal) 20 DAS, estimated as  $\mu$ g BHT equivalents per mg of fresh weight (FW). Each bar represents the average  $\pm$  SD of four independent measurements (n = 4). Different letters (a–d) indicate significant differences between treatments at p = 0.05 with a Tukey's HSD test.

UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS

SL cultivars (Figs. 5C,D), respectively. At the same time, dark-pigmented cultivars show no effect on scavenging rate after exposition (Figs. 5C,D).

## Discussion

The consumption of fruits and vegetables is related to the prevention of many diseases due to the antioxidant activity of plants' secondary metabolites (Jacobo-Velázquez et al., 2022). However, the postulated expansion of indoor farming agriculture, employing strictly controlled, stable grown conditions, results in the restriction of health-promoting compound levels, as they accumulate mostly in response to abiotic stresses. Thus, the aim of the study is to elucidate an efficient, easy-to-operate and non-invasive method to biofortify plant tissue with secondary metabolites intended to be applied within indoor farming. The proposed application is based on the short-term exposition of plants to low doses of UV-A radiation directly prior to harvest. We analysed the influence of UV-A treatment on two popular leafy species of lettuce and basil in the form of baby leaf (leafy greens) and microgreens, respectively. Lettuce and basil plants were grown in green- and redleaf cultivars.

Similar approaches have been previously analysed in paper of Ordidge et al. (2010), which evaluated the effect of plant cultivation in open-sided tunnels under a plastic film of three different UV light transparency. The mentioned authors, documented that the application of films with higher UV transparency induced accumulation of total phenolics and flavonoids (quercetin) in red lettuce, but was ineffective in the case of green lettuce cultivar. In the case of our study, we documented opposite results, as the green leaf cultivars of both lettuce (cv. Lollo Bionda) as well as basil (cv. Sweet Large) present significantly higher

responsiveness to UV-A-depended phenolic compounds synthesis than darkpigmented cultivars (Lollo Rossa and Dark Opal). In leaves, phenolics accumulation protects photosynthetic apparatus against UV damage, thus the green cultivars presented significantly lower TPC when grown without stressors such as UV, while it made them more vulnerable to UV-A exposition that activates phenolics synthesis and deposition. In the case of dark-pigmented cultivars UV-A is ineffective to stimulate further accumulation of TPC above the high, initial level. Moreover, the UV-A exposition exerted a negative influence on TPC, as a result of partial degradation of phenolics absorbing UV-A or restricted penetration of UV-A due to low doses applied. The latter explanation agrees with analyses of Qin et al. (2023), who documented that UV-A induced enhanced TPC accumulation in purple lettuce cultivars when applied at higher intensity, while plants grown under low-doses of UV-A showed control-like TPC. Also, Kang et al. (2022) documented that higher doses of UV-A light can improve TPC accumulation in basil.

In addition to TPC, we also analysed flavonoids concentration (TFC) as TPC and TFC synthesis share a mutual biochemical pathway. It has been documented that UV-mediated changes in phenolics and flavonoids levels may be attributed to its ability to induce the gene expression of phenylalanine ammonia lyase (PAL), a key enzyme involved in the first step of the phenylpropanoid pathway (Wong et al., 2020). Like phenolics, also flavonoids may benefit consumer health due to their anti-oxidative and presumed anti-carcinogenic effect (Rodriguez et al., 2014). Consequently, we also analysed the total antioxidant capacity of plants with and without UV-A extracts exposition. In a previous study (He et al., 2021) it has been reported that UV-A exposition of lettuce increased the radicalscavenging rate measured with DPPH, due to enhanced accumulation of TFC and TPC. Also, Lee et al. (2013) documented that plants of sowthistle (Ixeris dentata Nakai) exposed to UV-A for 5 days presented 50% higher flavonoid content than the control. In this study, we documented that the accumulation pattern of flavonoids follows that of phenolics, namely UV-A exposition exerts a stimulating effect in green leaf cultivars of lettuce and basil, while in dark-pigmented cultivars we observed slightly decreased the TFC. Moreover, we noted that the TAC of plant extract is strictly related to actual TFC and TPC, and consequently was significantly enhanced in green cultivars: Lollo Bionda and Sweet Large. Basil cv. Dark Opal presents a consequent decrease of TAC. However, an exception to this was red leaf lettuce - Lollo Rossa, which shows a little TAC increase after UV-A treatment. As we documented that the Lollo Rossa cultivar showed decreased levels of both TPC and TFC it might be other antioxidant compounds such as anthocyanins, that accumulated after UV-A treatment that enhanced TAC.

Taken together, our results are in accordance with Lee et al. (2014) that low-dose documented UV-A treatment enhanced phenolic compound and antioxidant properties in lettuce particularly between days 1 and 4 after exposure. However, prolonged UV-A exposition attenuated this effect and concentration of antioxidative compounds decreased to the control level. The authors explain that such an effect may be a consequence of continuous quenching of ROS with antioxidant phytochemicals. Such a reaction may be expected as increased accumulation of UV-A highly absorbing compounds such as phenolics and flavonoids make more the UV radiation to be absorbed and generates ROS. Presumably, such a response was also a consequence of the unresponsiveness of darked-pigmented cultivars of lettuce and basil to induce further antioxidant accumulation, as they already contain high TPC and TFC.

## Conclusions

The results demonstrate that low-dose (9 W m<sup>-2</sup>) UV-A short-term (4 days, total 15h) exposure applied within a red-greenblue light spectrum background allowed to induce accumulation of healthpromoting phytochemicals such as phenolics and flavonoids and overall antioxidants capacity measured as ability to scavenging the DPPH radical. Analyses were conducted on lettuce and basil plants, grown in short crop time - baby *leaf* and *microgreens*, respectively. However, the positive effect was observed only for green leaf cultivars - Lollo Bionda of lettuce and Sweet Large of basil. Dark-pigmented cultivars presented significantly higher antioxidant compounds under the control spectrum (RGB), whereas after UV-A treatment presented both cultivars slightly decreased of phenolic and flavonoids. The study provides an approach for indoor farming spectrum optimization, mostly devoid of UV components, to improve the quality of plant leafy products.

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UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS

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UV-A RADIATION FOR BIOFORTIFICATION OF LETTUCE AND BASIL PLANTS