Optimal red: Blue ratio in led lighting for nutraceutical indoor horticulture

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TITLE: OPTIMAL RED:BLUE RATIO IN LED LIGHTING FOR NUTRACEUTICAL INDOOR HORTICULTURE.

RUNNING TITLE: LED SPECTRA FOR NUTRACEUTICAL PLANTS

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ABSTRACT

In recent years, the interest toward the applicability of Light-Emitting Diode (LED) lights for indoor cultivation has significantly grown. The present work addressed the physiological and

phytochemical plant responses to LED lights in indoor cultivation of leafy and fruit vegetable crops (namely sweet basil, *Ocimum basilicum* L.; and Strawberry, *Fragaria* x *Ananassa*), with the final aim of improving both productivity and nutritional quality. Artificial light treatments were applied in a multi-sectorial growth chamber equipped with lamps with different light incidence and spectra (with red:blue ratio ranging 0.7 to 5.5). In all experiments, increased plant biomass, fruit yield and energy use efficiency (EUE) were associated to LED treatments, confirming the superiority of LED compared to the traditional fluorescent lamps. Interestingly, LED lighting enabled to increase antioxidant compounds and reduce nitrates content in basil leaves. A spectral red:blue ratio of 0.7 was necessary for proper plant development and improved nutraceutical properties in both crops.

Keywords: Sweet basil (*Ocimum basilicum* L.); Strawberry (*Fragaria* x *Ananassa*); antioxidants; energy use efficiency.

1. INTRODUCTION

Artificial lighting is gaining relevance in agriculture, since it enables intensification of production, improves quality, and allows cultivation wherever natural light is not sufficient (e.g. northern latitudes, indoor cultivation). Light-Emitting Diodes (LEDs) were introduced in plant cultivation in the 2000s as a more efficient light source, compared to fluorescent lighting and High Pressure Sodium (HPS) lamps. LEDs are expected to reduce the electricity costs of lighting and cooling because they have a higher efficiency of converting electric power to light power and require lower cooling loads than conventional light sources. Furthermore, it is easier to manipulate the spectral distribution of LEDs (Goto, 2012). The high intensity LED lamps are a potential alternative to current lighting technology due to their long functional life, low operating temperatures, low energy consumption and selective spectral output (Hernàndez and Kubota, 2012). Compared to commonly used sources of light, LED has features such as numerous types of wavelength, energy saving, short response time,

small size, light weight and less heat production (Zhang et al., 2011). In recent years, several types of LED-based lamps became available for commercial plant production.

Artificial light supply presents an additional cost compared to cultivation under natural sunlight. As a result, the problem of both yield and quality of the produce becomes extremely relevant. Consistently, when considering the applicability of LED, maximizing productivity is an important issue: here the grower is always challenged to prevail economically within the limits of plant growth and cost reduction (Domurath et al., 2012). Although different crops require various light regimes, it has been confirmed that the optimal ratio between blue and red light is of great relevance in determining yield (Tarakanov et al., 2012). Moreover, given that LED lamps may be placed within plant canopy, previous studies claimed that increased crop growth is also related to improved light interception rather than increased photosynthetic rates (Hogewoning et al., 2012). A great opportunity for the financial sustainability of artificial lighting is provided by the chance of quality improvement. Light is one of the most important variables affecting phytochemical concentration in plants (Kopsell and Kopsell, 2008). It has been reported that the use of LED lamps in lettuce cultivation positively affected growth and phytochemical traits (Li and Kubota, 2009). Other investigations showed the LED-induced increase of the anthocyanins in grapes (Kataoka et al., 2003) and lettuce leaves (Tsormpatsidis et al., 2008), carotenoids in tomato fruits and anthocyanins in tomato leaves (Giliberto et al., 2005) and vitamin C in lettuce leaves (Ohashi-Kaneko et al., 2007). Concurrently, given that light is one factor affecting some enzymes activation (e.g. nitrate reductase), LED lighting has also been reported to reduce NO_3^- in Indian mustard (*Brassica juncea* L.) (Tarakanov et al., 2012). Consumption of excess nitrate is considered to be dangerous for infants and a potential health hazard to older children and adults. This is because nitrate can be converted to nitrite in the gut, and nitrite can bind to hemoglobin thus preventing the blood from carrying enough oxygen or, in presence of ammine, may generate nitrosamines, known to have carcinogenic activity (Speijers, 1996). The reduction of anti-nutritional compounds in horticultural produce, such as nitrates in leafy vegetables, has therefore become an important objective in agricultural research (Milner, 2002).

The possibility that combinatorial light regimes may help to optimize growth and control developmental transitions makes the implementation of LED technology particularly attractive to the design of controlled environments targeted to plant production (Samuolienè et al., 2010). This study aims to assess the differences in plant growth performance under traditional fluorescence lamps and LED light system, leading to a more complete understanding of the physiological and phytochemical plant response to the spectral components of light, and finally detecting the optimal LED light spectra composition for obtaining nutraceutical horticultural products. Therefore, starting from already tested spectra, (Samuolienè et al., 2012; Yoshida et al., 2012), the experiments were conducted to investigate further spectral applications for improved yield and quality in crops whose product is represented by either leaves (basil, *Ocimum basilicum* L. cv Genovese) or fruits (strawberry, *Fragaria x Ananassa* cv. Elsinore). The aim of the work was therein to identify optimal spectral composition for obtaining food with improved nutraceutical properties.

2. MATERIALS AND METHODS

2.1. Plant Material and Growth Conditions

Four experiments were consecutively conducted in a 9 m^2 walk-in growth chamber at the Department of Agricultural Sciences (DipSA) of the University of Bologna, Italy. Fifteen days old plantlets of basil (*exp. 1# and 2#*) and strawberry (*exp. 3# and 4#*) were transplanted into plastic 1 liter-pots (1 plant per pot) filled with a volcanic growing media (particle size 0-15mm; pH 7; humidity 18.8%; EC 0.23 mS cm^{-1}; total organic carbon 2.2% DM; N 0.42% DM; P 0.12 % DM; K^+ 3.7 % DM; Ca^{2+} 2.5 % DM; Mg^{2+} 1.2 % DM; Fe^{2+} 2.6 % DM). Plants were automatically drip irrigated three times per day to ensure adequate substrate moisture. Planting density was respectively 24 and 9 plants m^{-2}, for basil and strawberry. Fertigation was carried out once a week by adding to the irrigation water at the following concentrations: N-NO_3^- : 6.0 mM; N-NH_4^+:1.0 mM; PO_4^{3-} : 3.0 mM; K^+: 4.0 mM; SO_4^{2-} : 7.0 mM; Ca^{2+} : 5.0 mM; Mg^{2+} : 4.0 mM; microelements in traces. The growth chamber was automatically regulated at 21±2°C, 55-70% of humidity and 450 ppm CO_2. The experiments were

closed when commercial harvest was reached, at 31 and 56 Days After Transplanting (DAT) in basil and strawberry, respectively.

2.2 Treatments and Experimental Design

Different artificial light treatments (16/8 h light/dark) were applied by dividing the growth chamber into separate sectors (on shelves fixed on the chamber walls and sealed using white lightproof sealed walls, each 0.3 m^2) and placing lamps with different spectrum and same photosynthetic photon flux density (PPFD) over the canopy (measured on top leaves, lamps at distance of 10 cm) in measure of 200 μmol m^{-2} s^{-1}. Five treatments (each of them replicated in four sectors, on 1.2 m^2), derived from the combination of 4 different LED lamps ($Led_{0.7}$, $Led_{1.1}$, $Led_{1.5}$, $Led_{5.5}$) and a fluorescent light (CK as a control) (TL-D 90 De Luxe 58W 950, Philips, Amsterdam, The Netherlands), were compared (see specs in **Table 1** and **Fig. 1**). Ratio between red and blue portions of the spectrum were calculated by defining the surfaces of the whole spectrum within the red (635-700 nm) and the blue (450-490 nm) wavelengths. One LED lamp was used in each 0.3 m2 sector, kindly provided by Bulbo (Bologna, Italy, further specifics on www.bulbolight.com).

Table 1. Different LED light spectrum components used in the two experiments.

LED components			**Energy**	**Spectrum**			**Treatment Code**
Red (%)	**Blue (%)**	**White (%)**	**W plant^{-1} h^{-1}**	**Red (%)**	**Blue (%)**	**Red:Blue ratio**	
40	40	20	1.5	25.1	37.7	0.7	$Led_{0.7}$
40	30	30	1.5	24.0	22.2	1.1	$Led_{1.1}$
50	20	30	1.5	29.6	19.5	1.5	$Led_{1.5}$
90	10	0	1.5	39.9	7.3	5.5	$Led_{5.5}$
-	-	-	5.8	6.8	14.1	0.5	CK

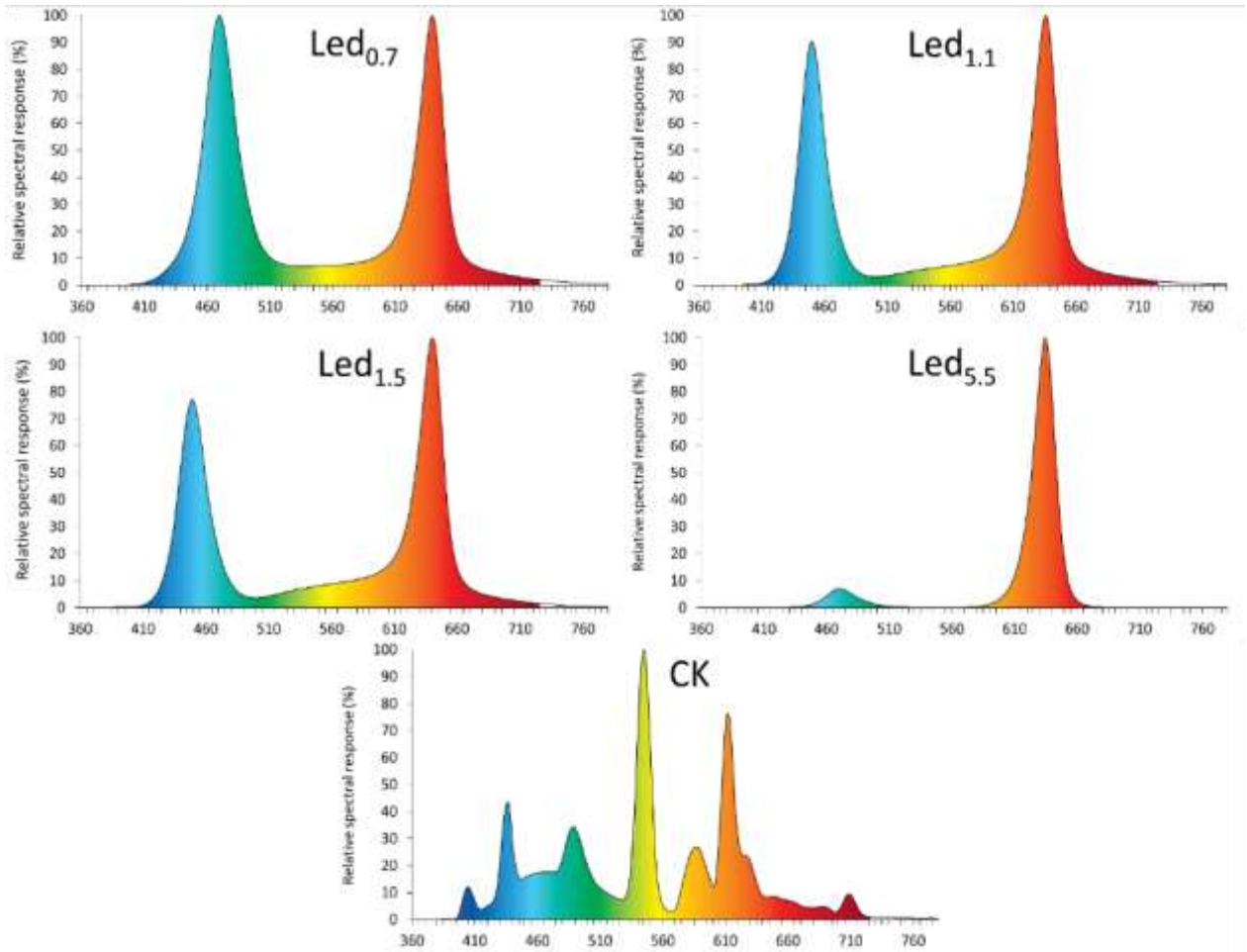

Figure 1. Wavelength Spectra of the different lamps used in the experiments (see specs in Table 1).

2.3 Vegetative and physiological measurements

At harvest time, total plant fresh weight (FW) was measured in all experiments and total fruit yield determined in strawberry. Additionally, basil leaves and strawberry fruits were immersed in liquid nitrogen and kept at -80 °C for biochemical analysis. Measurements of leaf gas exchanges were performed for both plant species on attached leaf samples using a CIRAS-2 (PPSystem, Hitchin, UK) infrared gas analyser (closed system) with a Parkinson's Automatic Universal Leaf Cuvette (air inside the cuvette with the following characteristics: 26°C, CO_2 450 ppm and 300 cm^3 min^{-1} flow rate) equipped with 18-mm diameter, 2.5-cm^2 area cuvette inserts. Net photosynthesis (*A*) was measured at 14 DAT on four completely unfolded leaves per each plant species and treatment. Energy use

efficiency (EUE) was determined according to the crop cycle length and the final FW and fruit yield, related to the lamps' power consumption and expressed as g kW^{-1}.

2.4 Biochemical determination

2.4.1 Extraction of phenolic compound

5g of basil leaf and strawberry fruit frozen samples were thawed at room temperature and homogenized with 20mL of methanol/H_2O/acetone (60:30:10 v/v/v) (Hartmann et al., 2008). Each mixture was centrifuged (ALLEGRA™ 25R Centrifuge, BECKMAN, El Cajon, CA, USA) at 15.300 xg for 10 minutes and the supernatant was collected. The extraction was repeated one time and the final extract (10 mL) was used for the determination of total phenolic and flavonoid content and total antioxidant capacity.

2.4.2 Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) was determined according to Folin-Ciocalteau colorimetry method (Giusti and Wrolstad, 2002). Briefly, 100 µL of extracted sample was mixed with 1.5 mL of distilled water in 2.5 mL plastic cuvette followed by 100 µL of Folin-Ciocalteau phenolic reagent and incubated for 5 minutes at room temperature. After mixing, 300 µL of 20% sodium carbonate were added and mixed thoroughly then incubated for 2 h at room temperature. The sample absorbance was measured at 765 nm by spectrophotometer (DU530® life science UV/VIS spectrophotometer, BECKMAN, El Cajon, CA, USA). All samples were measured in duplicate and the total phenolic content was expressed as gallic acid equivalent in milligram per g of fresh weight of basil leaves and strawberry fruits. Total flavonoid content (TFC) was determined by aluminium chloride colorimetric assay (Zhishen et al.,1999). Briefly, 750 µL of extracted sample was mixed with 750 µL of distilled water, and 75 µL of 5% $NaNO_2$ was added in a 2.5 mL plastic cuvette. After 6 minutes, 150 µL of 10% $AlCl_3$ was added, followed by 500 µL of 1 M NaOH after 5 minutes. Then the sample absorbance was measured at 510 nm by spectrophotometer and the calibration was carried out by a standard curve

of catechin (5, 10, 20, 25, and 50 ppm). The results were expressed as mg of catechin equivalents per grams of fresh weight of basil leaves and strawberry fruits.

2.4.3 Total Antioxidant Capacity

Total antioxidant capacity was measured by Ferric Reducing Antioxidant Power (FRAP) assay (Benzie and Strain,1999) after some modification (Aaby et al., 2007).

The composition of FRAP reagents was:

1. Acetate buffer 300 mM pH 3.6: 3.1 g sodium acetate tri-hydrate were added with 16 mL of glacial acetic acid and filled to the volume to 1 L with distilled water.
2. TPTZ (2, 4, 6-tripyridyl-s-triazine) 10 mM in 40 mM HCl
3. $FeCl_3.6H_2O$ 20 mM

The final FRAP reagent was prepared by mixing 1, 2 and 3 in the ratio of 10:1:1 at the time of use and covered with aluminium. Briefly, freshly prepared FRAP reagent (2.4 mL) was mixed with 80 μL of sample (0.1 g mL^{-1}) in duplicate. The mixture was equilibrated for 1 hour at room temperature before absorbance was measured at 593 nm. Aqueous solutions of Fe-(II) ($FeSO_4.6H_2O$) in the concentration range of 125-1250 μM were used for calibration of the FRAP assay. FRAP values were expressed as mmol Fe^{2+} kg^{-1} FW.

2.4.4 Nitrates content determination

Nitrates in basil leaf tissues were determined using an HACH DR/2000 spectrophotometer on samples extracts (Sah, 1994). The extraction was obtained by adding 0.5 g of sample dry matter to 100 ml of distilled water in a water bath at 100°C for 45 minutes. After cooling the samples, 3.5 g of activated charcoal powder were added to 50 ml extracts. The suspension was then filtered with Whatman filter paper (1 mm). Five millilitres of the filtered extract was subsequently mixed with Nitraver 5 Nitrate Reagent Powder (Hach, Loveland, CO, USA) and brought to 25 ml with distilled water. Extracts were read with the spectrophotometer at 500 nm. Results were expressed as mg kg^{-1} FW.

2.5 Statistical Analysis

The study employed a randomized block design with single experiments as elemental block and randomised design within the block with four replications and each replication represented by 8 and 3 plants (basil and strawberry, respectively). Data were analysed by two-ways ANOVA and the means were compared by Least Significance Difference (LSD), at 5% significance level.

3. RESULTS

3.1 Fresh Weight and Energy Use Efficiency Responses

In basil, leaf fresh weight was significantly lower in LED treatments having a red:blue ratio of 1.1, 1.5 and 5.5 compared to the CK, with the exception of $Led_{0.7}$ having a similar leaf biomass (**Table 2**). It was observed that decreasing the blue spectrum intensity, the basil fresh weight decreased almost constantly: while red:blue ratio increased by about 1.5, 2.0 and 8.0 folds, plant FW was diminished by 16%, 39% and 68% referring to the highest plant FW under the light $Led_{0.7}$ (**Fig. 2**). Consistently, greatest EUE performances were observed in plants grown under $Led_{0.7}$ resulting in a 5-fold increase in the biomass produced per kW consumed as compared to CK (**Table 2**).

In strawberry, higher plant fresh weight was observed in all LED treatments, resulting in a biomass increase up to 20 g plant^{-1} as compared to CK (**Table 2**). EUE was enhanced in all LED treatments, being significantly different from CK (**Table 2**). The greatest strawberry fruit production (g fruit plant^{-1}) was observed in $Led_{0.7}$ and $Led_{1.1}$ treatments (**Table 2**), both achieving more than a 3-fold higher productivity as compared to CK and $Led_{5.5}$ treatments. LED treatments resulted in greater fruit production also if referred to their power consumption: a more balanced spectral composition (including red, blue and white components) resulted in higher EUE with an average of 26.9 g kw^{-1} ($Led_{0.7}$, $Led_{1.1}$ and $Led_{1.5}$ vs 4.6 g kw^{-1}) ($Led_{5.5}$ and CK) (**Table 2**).

Table 2. Foliar and fruit biomass (FW, g plant^{-1}) and Energy Use Efficiency (EUE, g kW^{-1}) in plants of basil (*Ocimum basilicum* L.) and strawberry (*Fragaria* x *Ananassa*) as affected by light (see specs in Table 1 and Fig. 1). Mean values. Different letters indicate significant differences at P≤0.05.

Light treatment	**Basil**				**Strawberry**							
	Leaves				**Leaves**				**Fruits**			
	FW (g plant^{-1})		**EUE (g kW^{-1})**		**FW (g plant^{-1})**		**EUE (g kW^{-1})**		**FW (g plant^{-1})**		**EUE (g kW^{-1})**	
Led$_{0.7}$	**58.1**	**a**	**83.0**	**a**	25.4	b	**36.2**	**a**	**19.0**	**a**	**27.1**	**a**
Led$_{1.1}$	48.6	b	69.4	b	**27.9**	**ab**	**39.9**	**a**	**23.0**	**a**	**32.9**	**a**
Led$_{1.5}$	35.7	c	44.6	c	**35.8**	**a**	**44.8**	**a**	**16.5**	**ab**	**20.6**	**ab**
Led$_{5.5}$	18.5	d	23.2	d	24.7	b	**35.0**	**a**	5.3	b	6.6	b
CK	**50.9**	**a**	17.6	d	15.8	c	5.5	b	7.8	b	2.7	b

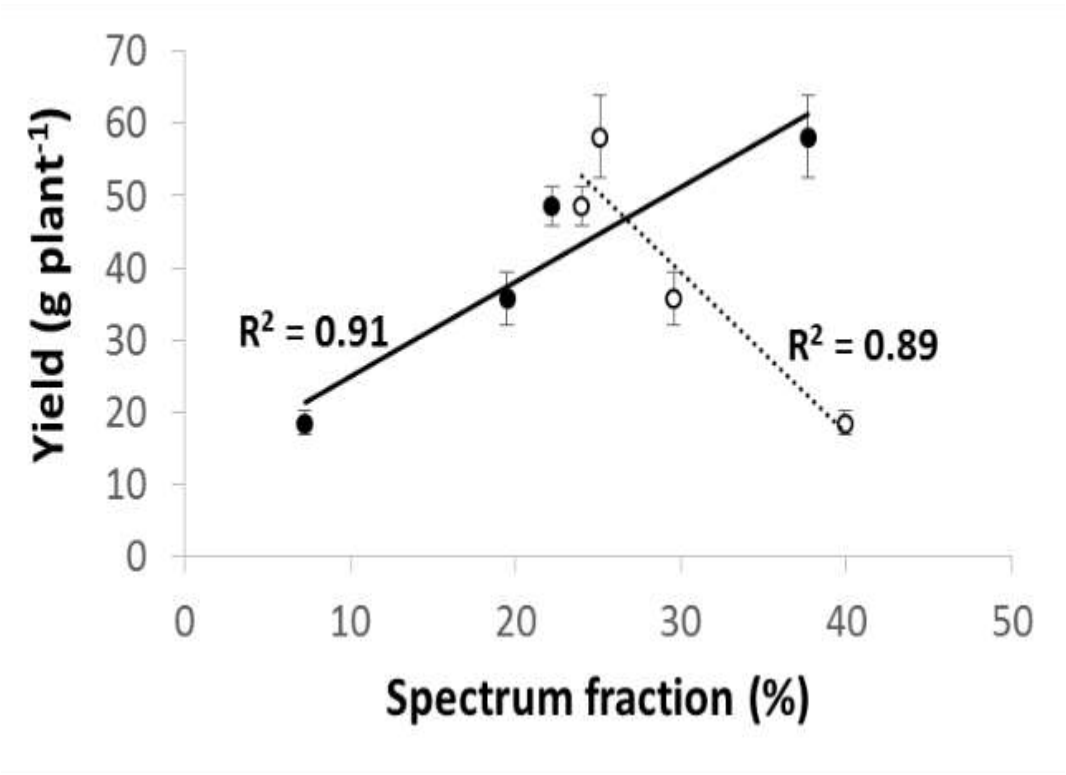

Figure 2. Linear relationship between yield (g plant^{-1}) and the spectral fraction of blue (closed circles) and red (open circles) in basil (*Ocimum basilicum* L.). Mean values ± SE, n=24.

3.2 Assimilation Rates

No significant difference in *A* of basil leaves between LED treatments and CK were observed, with the exception of $Led_{5.5}$ where *A* was significantly lower than CK (**Fig. 3.A**). In strawberry plants, there were no significant differences in A between LED treatments and the control CK, though a significant reduction in A was observed under $Led_{5.5}$ compared to $Led_{0.7}$ (**Fig. 3.B)**.

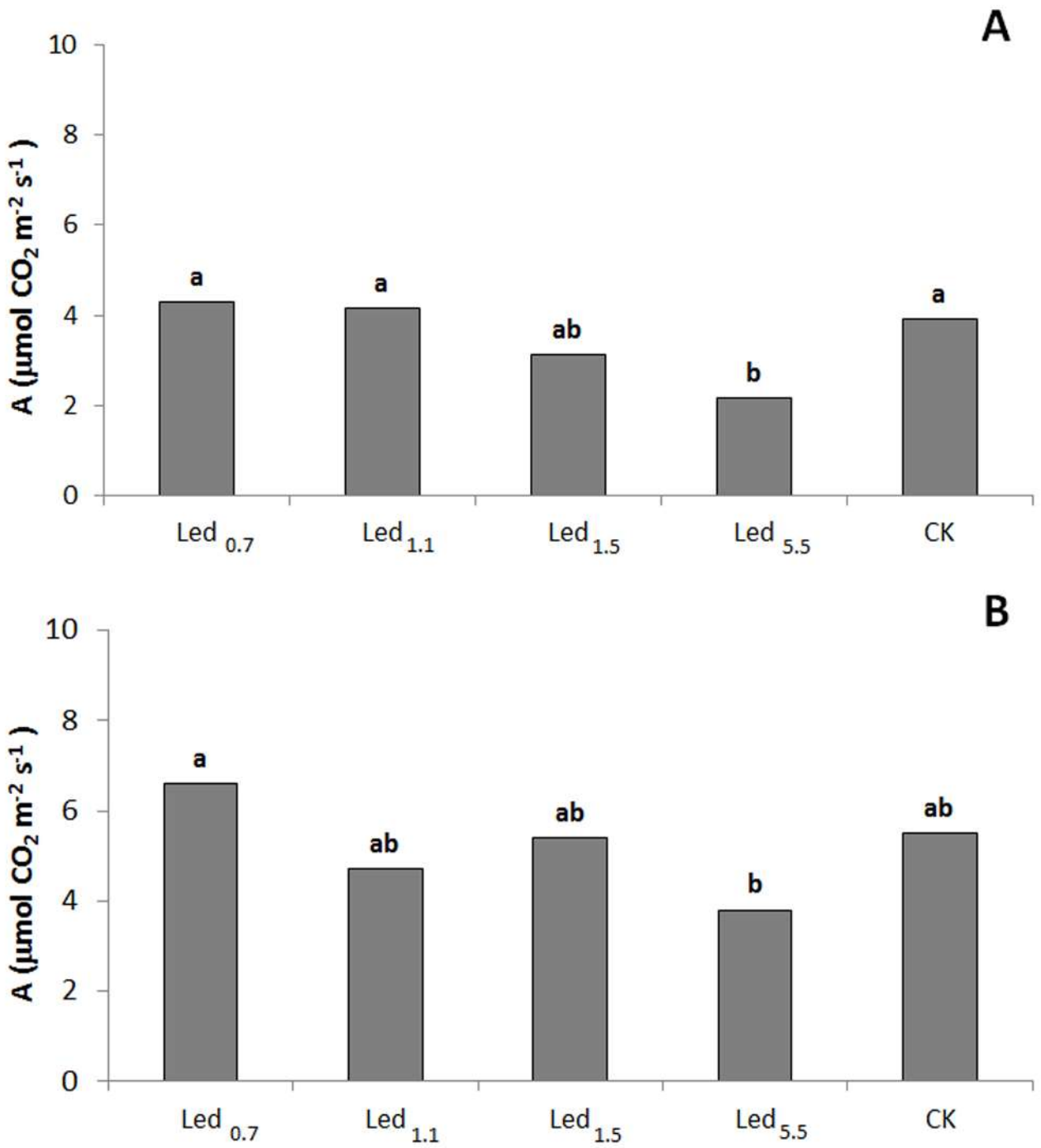

Figure 3. Assimilation rate (*A*, µmol CO_2 m^2 s^{-1}) in plants of basil (*Ocimum basilicum* L., **A**) and strawberry (*Fragaria* x *Ananassa*, **B**) as affected by light (see specs in table 1). Mean values. Different letters indicate significant differences at $P \leq 0.05$.

3.4 Antioxidant Capacity, Polyphenols and Flavonoids

In basil, significant increases in antioxidant capacity, phenolics and flavonoids contents were observed in all LED treated leaves compared to the CK ones (**Table 3**). In strawberry, only few significant differences were detected in the FRAP assay: the lowest antioxidant content was found in plants grown under $Led_{0.7}$ (-4% compared to $Led_{1.5}$, $Led_{5.5}$ and CK). No differences were detected in terms of phenolic content, while in the flavonoid content a significant increase was observed in fruits grown under CK (+6% as compared to all other treatments **Table 3**).

Table 3. Antioxidant capacity (FRAP), phenolics and flavonoids contents in basil (*Ocimum basilicum*) leaves and strawberry (*Fragaria* x *Ananassa*) fruits as affected by light (see specs in table 1). Mean values. Different letters indicate significant differences at P≤0.05.

	Basil								**Strawberry**							
	Leaves								**Fruits**						**Leaves**	
Light treatment	**FRAP (mmol Fe^{2+} kg^{-1} FW)**		**TPC (GA g^{-1} FW)**		**TFC (mg CE g^{-1} FW)**		**NO_3 (mg kg^{-1} FW)**		**FRAP (mmol Fe^{2+} kg^{-1} FW)**		**TPC (GA g^{-1} FW)**		**TFC (mg CE g^{-1} FW)**		**NO_3 (mg kg^{-1} FW)**	
$Led_{0.7}$	**4.2**	**a**	**12.5**	**a**	**3.5**	**a**	658	b	4.3	b	1.4	a	0.31	b	996	b
$Led_{1.1}$	**4.3**	**a**	**12.3**	**a**	**3.6**	**a**	528	b	**4.4**	**ab**	1.3	a	0.32	b	**1237**	**a**
$Led_{1.5}$	**4.2**	**a**	**12.4**	**a**	**3.4**	**a**	718	b	**4.5**	**a**	1.4	a	0.32	b	1094	b
$Led_{5.5}$	**4.1**	**a**	**11.6**	**a**	**3.2**	**a**	**984**	**a**	**4.5**	**a**	1.4	a	0.32	b	949	b
CK	3.6	b	8.9	b	2.1	b	**1046**	**a**	**4.5**	**a**	1.3	a	**0.34**	**a**	**1276**	**a**

3.5 Nitrates Content

Highest nitrates contents in basil leaves were associated with CK and $Led_{5.5}$ (mean value 1015 mg kg^{-1} FW) treatments **(Table 3)**. Nitrates content was significantly lower in basil plants grown under $Led_{0.7}$, $Led_{1.1}$ and $Led_{1.5}$ (mean value 635 mg kg^{-1} FW). As regard strawberry leaves, lower values were observed in $Led_{0.7}$, $Led_{1.5}$ and $Led_{5.5}$ (mean value 1013 mg kg^{-1} FW) as compared to $Led_{1.1}$ and CK (mean value 1256 mg kg^{-1} FW) **(Table 3)**.

4. DISCUSSION

4.1 LED influence on plant growth and yield

In strawberry, the adoption of LED lights resulted in increased fruit and leaves biomass production, wheras leaf yield of basil was similar to CK in $Led_{0.7}$, while decreased in the other LED treatments (**Table 2**). In strawberry, the beneficial effects of LED lighting on plant yield versus the conventional fluorescent lamps has been described by Yoshida et al. (2012), mainly as a consequence of anticipated flowering and shortened vegetative growth period. Improved vegetative growth was also associated with LED lighting on tomato and cucumber (Hogewoning et al., 2012) and on arabidopsis (Norling et al., 2012). Among LED treatments considered in the present study, yield was increased to a greater extent in plants grown under $Led_{0.7}$ (**Table 2**), confirming that the proper balancing of red and blue components of the light spectrum would be beneficial to plants (Hogewoning et al., 2012).

It is important to note that, in all experiments, CK presented the lowest EUE performances, as a consequence of its higher energy consumption (up to almost 4 times more than the LED lamps) (**Table 2**) (Goto, 2012). Consistently, the present work confirmed the energetic efficiency superiority of LED compared to the traditional fluorescent lamps, enabling an increase of 3 to 9 folds productivity per unit energy used (being EUE maximised in $Led_{0.7}$) (**Table 2**). Overall, productivity and energy use efficiency was ultimately correlated with specific LED features (spectra), rather than the LED technology per se, indicating the relevance of optimal spectral selection.

4.2 How do plants respond to different spectral compositions?

Basil plants performed best when the blue ratio was slightly predominant ($Led_{0.7}$) (**Table 2**). Under the same light, blooming in strawberry was anticipated (one week ahead, data not shown), and this turned out to result in greater fruit yield (Samuolienè et al., 2010; Yoshida et al., 2012), as compared with CK. Researches (Schamp et al., 2012) conducted on Ghent azalea (*Rhododendron* x *gandavense*), also showed an advance in blooming and in flower size when blue proportion was

enhanced from 9 to 18 μmol m^{-2} s^{-1}. Nevertheless, strawberry fresh weight was further improved (**Table 2**) under $Led_{1.1}$ and $Led_{1.5}$, confirming that a species-specific mixture of red and blue spectral components is necessary for proper plant development (Samuolienè et al., 2010). The reduction of fruit yield in plants grown under red LED light ($Led_{5.5}$) or CK (**Table 2**) may be associated with reduced flowering and fruiting as a consequence of insufficient blue light fraction (Yoshida et al., 2012).

The effect of the blue component in promoting plant yield has been addressed in a range of recent reports, although often with controversial results. A reduction was reported by Tarakanov et al. (2012) in basil yield when plants were grown under a spectrum with a prevalent red fraction, similar to $Led_{5.5}$. Furthermore, the improvement on the biomass of Welsh onion (*Allium fistulosum* L.) shoot with blue, rather than red and green, overnight supplemental lighting was reported by Sase et al. (2012). On the other hand, strawberry vegetative growth increased when blue percentage was lower though still balanced by a white component (Samuolienè et al., 2010). More recently, the increases in plant growth to white and red components rather than the green and blue fractions in *Tetraselmis suecica* has been documented (Abiusi et al., 2013). In the above experiments, the blue component and its ratio within the spectrum was positively correlated with leaf yield (**Fig. 2**) in basil.

Plant physiological and biochemical activities are strictly correlated with the quality of the incident light (Horton, 2000). Consistently, identification of the optimal spectral composition shall take into account how plant functions varied across light treatments.

It is widely known that red spectral regions of light have the strongest impact on the rates of photosynthesis in plants. Photosystems (PS) I and II absorb wavelengths around 650 nm (PSII) and 700 nm (PSI) (Schopfer and Brennicke, 2010). In the present study however, all species showed better photosynthetic performance when an additional proportion of blue light was present, namely under $Led_{0.7}$, $Led_{1.1}$, $Led_{1.5}$ and CK (**Fig. 3**). However, given the lower yield of photosynthesis under blue monochromatic light, it is still suggested to use a combination of red and blue spectral regions (Domurath et al., 2012). According to these results overall, the photosynthetic performances did not

affect plant biomass productivity itself, which was rather affected by spectral light composition and the balance between red and blue fractions (**Fig. 3**). Consistently, the increased crop growth under LED lighting should be related to improved light interception rather than increased photosynthetic rates (Hogewoning et al., 2012). Until now, the literature offers very few references to the "right" spectral composition, whose balance must necessarily be adapted to the crop's requirements and biochemical responses.

4.3 Optimal spectral compositions lead to improved nutraceutical properties of plant products

The most promising spectra identified for the crop productive performance, were also evaluated in order to understand their effect on plant biochemical composition that represents a customer added value. Interestingly, regarding basil, all LED treatments lead to an increase in the whole antioxidant pool (**Table 3**). Antioxidant capacity was increased in measure of about a fifth in basil grown under LED as compared with CK. Phenolic compounds were significantly higher in basil leaves grown under LED as compared to CK. The increase (+70%) of flavonoids in basil leaves observed in all LED treatments as compared to CK may be related to the stress caused by the different light quality (Winkel-Shirley, 2002). The supplementary red LED lighting improves total antioxidants capacity, phenolics and anthocyanins in microgreens (Samuolienè et al., 2012), while Goto (2012) referred to an enhancement of anthocyanins in lettuce grown under blue LED. From a physiological perspective, the visible component of the spectra was shown to activate proanthocyanidins biosynthesis in grape berries (Koyama et al., 2012). Excess light has been also shown to activate flavonoids accumulation in *Ligustrum* sp., under a coordinate control system between hydroxycinnamate and flavonoid pathways (Tattini et al., 2004).

The use of LED lights for indoor cultivation of arugula, has been shown to cause an overall increase (+22%) in the flavonoids concentration as compared to HPS lighting. However, LED did not positively affect strawberry plant in terms of antioxidant compounds. Overall, significantly higher

flavonoids content were detected in CK strawberry treated plants, which probably affect also total antioxidant pool **(Table 3)**.

Consistent with the increase in antioxidants, a general decrease in the NO_3^- concentration in basil green tissue was associated with LED as compared to CK. In plants, nitrate accumulation is counteracted by the activity of nitrate reductase, an enzyme regulated by nitrate availability (Crawford, 1995), plant nutritional status (Hunt and Mcneil, 1998), and light (Becker et al., 1992). In open field conditions, nitrate concentration in leaves usually declines during the day from sunrise to sunset (Orsini and De Pascale, 2007). Nitrogen concentration of plants usually declines during growth even under sufficient N supply (Kage et al., 2002). Further researches should address the understanding if the improved light spectral quality associated to LED lighting may have been responsible of the higher nitrate reductase activity, which resulted in lower NO_3^- concentration in leaves. In basil plants grown using $Led_{5.5}$, plant physiological functions were compromised by the non-suitable spectrum (as appearing in plant FW, **Table 2** and **Fig. 2**, and photosynthesis, **Fig. 3.A**), and therein a decrease in nitrate reductase activity would also be observed.

5. CONCLUSIONS

This study addressed the applicability of LED lights for indoor cultivation of leafy and fruit crops (namely sweet basil and strawberry). Through a range of analyses (addressing morphological, physiological and biochemical elements), it was possible to determine the most suitable spectra for these crop species, namely with a red:blue ratio of 0.7. Consistently, LED lights improved crop features (ranging from yield and energy use efficiency, to antioxidant compounds with nutraceutical properties) and reduced unwanted compounds (e.g. nitrates), as summarised in **Fig. 4**, where $Led_{0.7}$ is compared with the relative fluorescent light control (CK).

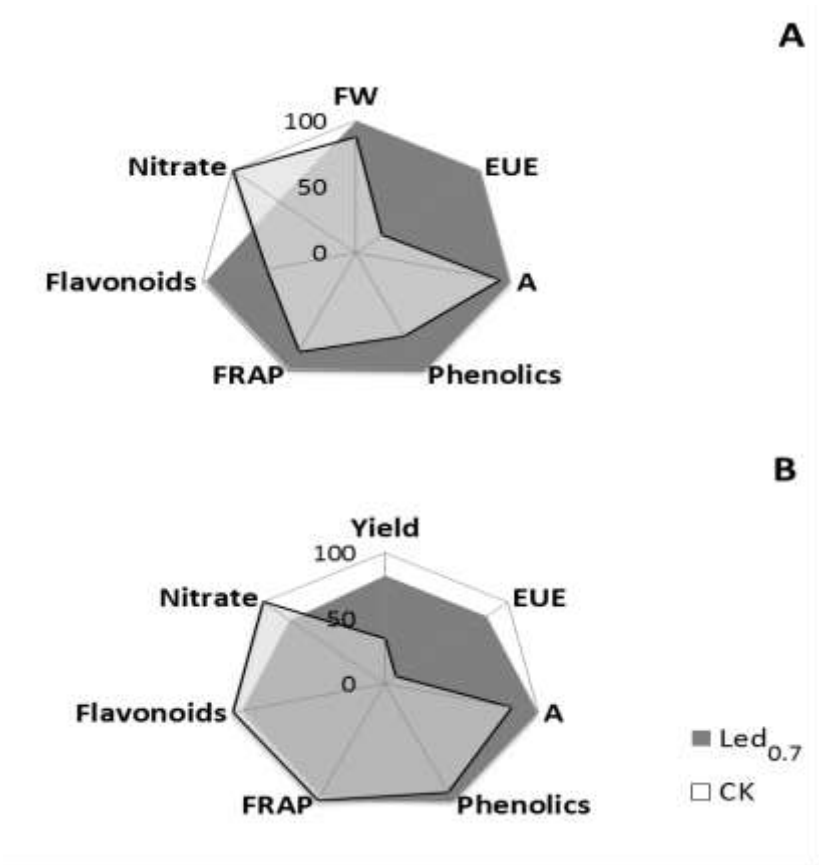

Figure 4. Representation summarizing the various parameters that have been recorded in this study. Best performant LED light ($Led_{0.7}$) is compared for each species with the relative fluorescent control (CK). Relative performances (as compared to maximum detected value) in plants of basil (*Ocimum basilicum* L., **A**) and strawberry (*Fragaria* x *Ananassa*, **B**) as affected by light (see specs in Table 1).

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7. REFERENCES

Aaby, K., Wrolstad, R.E., Ekeberg, D., Skrede, G., 2007. Polyphenol composition and antioxidant activity in strawberry purees; impact of achene level and storage. J. Agric. Food Chem 55, 5156-5166.

Abiusi, F., Sampietro, G., Marturano, G., Biondi, N., Rodolfi, L., D'Ottavio, M., Tredici, M.R., 2013. Growth, photosynthetic efficiency, and biochemical composition of Tetraselmis suecica F&M-M33 grown with LEDs of different colors. Biotechnol Bioeng 111, 956-964.

Becker, T.W., Caboche, M., Carrayol, E., Hirel, B., 1992. Nucleotide sequence of tobacco cDNA encoding plastidic glutamine synthetase and light inducibility, organ specificity and diurnal rhytmicity in the expression of the corresponding genes of tobacco and tomato. Plant Mol. Biol. 19, 367-379.

Benzie, F.F., Strain, J.J., 1999. Ferric Reducing/Antioxidant Power assay: Direct Measure of Total antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement of Total Antioxidant Power and Ascorbic Acid Concentration. Meth. Enzymol.. 299,15-23

Crawford, N.M., 1995. Nitrate: Nutrient and Signal for Plant Growth. Plant Cell 7, 859–868.

Domurath, N., Schroeder, F-G., Glatzel, S., 2012. Light Response Curve of Selected Plants under Different Light Conditions. Acta Hortic. 956, 291-298.

Giliberto, L., Perrota, G., Pallara, P., Weller, J.L., Fraser, P.D., Bramley, P.M., Fiore, A., Tavazza, M., Giuliano, G., 2005. Manipulation of blue light photoreceptor cryptochrome 2 in tomato affects vegetative development, flowering time, and fruit antioxidant content. Plant Physiol. 137, 199-208.

Giusti, M., Wrolstad, R.E., 2002. Determination of Total Phenolics. Curr. Protoc. Food Analyt. Chem. I1.1.1-I1.1.8.

Goto, E., 2012. Plant production in a closed plant factory with artificial lighting. Acta Hortic. 956, 37-49

Hartmann, A., Patz, C.D., Andlauer, W., Dietrich, H., Ludwig, M., 2008. Influence of processing on quality parameters of strawberries. J. Agric. Food Chem. 56, 9484-9489.

Hernàndez, R., Kubota, C., 2012. Tomato seedling growth and morphological responses to supplemental LED lighting red:blue ratios under varied daily solar light integrals. Acta Hortic. 956, 187-194.

Hogewoning, S.W., Trouwborst, G., Meinen, E., van Ieperen, W., 2012. Finding the optimal growth-light spectrum for greenhouse crops. Acta Hortic. 956, 357-363.

Horton, P., 2000. Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. J. Exp. Bot. 51, 475-485.

Hunt, J.E., McNeil, D.L., 1998. Nitrogen status affects UV-B sensitivity of cucumber. Aust. J. Plant Physiol. 25, 79-86.

Kage, H., Alt, C., Stutzel, H., 2002. Nitrogen concentration of cauliflower organs as determined by organ size, N supply, and radiation environment. Plant Soil 246, 201-209.

Kataoka, I., Sugiyama, A., Beppu, K., 2003. Role of ultraviolet radiation in accumulation of anthocyanin in berries of 'Gros Colman' grapes (*Vitis Vinifera* L.). J. JPN. Soc. Hortic. Sci 72, 1-6.

Kopsell, D.A., Kopsell, D,E., 2008. Genetic and environmental factors affecting plant lutein/zeaxantin. Agro Food Ind. Hi. Tech. 19, 44-46.

Koyama, K., Ikeda, H., Poudel, P.R., Goto-Yamamoto, N., 2012. Light quality affects flavonoid biosynthesis in young berries of Cabernet Sauvignon grape. Phytochem., 78, 54-64.

Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby leaf lettuce. Environ. Exp. Bot. 67, 59-64.

Milner, J., 2002. Functional foods and health: A US perspective. Br. J. Nutr. 88, S151-S158.

Norling, C.L., Wiggins, H.N., Crawford, J.I., Wotton, A.W.M., 2012. Flexible spectra LED arrays for sole source lighting and growth comparisons with conventional high pressure discharge lighting using Arabidopsis thaliana. Acta Hortic. 956, 113-120.

Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., 2007. Effect of light quality on growth and vegetable quality in leaf lettuce, spinach and komatsuna. Environ. Control Biol. 45, 189-198.

Orsini, F., De Pascale, S., 2007. Daily variation in leaf nitrate content of two cultivars of hydroponically grown basil. Acta Hortic. 747, 203-210.

Sah, R.N., 1994. Nitrate-nitrogen determination: a critical review. Commun Soil Sci Plant Anal. 25, 2841-2869.

Samuolienė, G., Brazaitytė, A., Urbonaviciutė, A., Sabajevienė, G., Duchovskis, P., 2010. The effect of red and blue light component on the growth and development of frigo strawberries. Zemdirbyste-Agric. 97, 99-104.

Samuolienė, G., Brazaitytė, A., Sirtautas, R., Sakalauskiene, S., Jankauskiene, J., Duchovskis, P., 2012. The impact of supplementary short-term Red LED lighting on the antioxidant properties of microgreens. Acta Hortic. 956, 649-655.

Sase, S., Mito, C., Okushima, L., 2012. Effect of Overnight Supplemental Lighting with Different Spectral LEDs on the Growth of Some Leafy Vegetables. Acta Hortic. 956, 327-333.

Schamp, B., Pauwels , E. and Gobin, B., 2012. Developing led light recipes for multi-layering systems: LED as an alternative for HPS in forcing of rhododendron. Acta Hortic. 956: 121-128

Schopfer, P., Brennicke, A., 2010. Pflanzenphysiologie. Spektrum Akademischer Verlag, Heidelberg. Seventh edition.

Speijers, G.J.A., 1996. Nitrite (and potential endogenous formation of N-nitroso compounds). In: Toxicological evaluation of certain food additives and contaminants in food, WHO Food Additives Series, Ginevra 35, 269-323.

Tarakanov, I., Yakovleva, O., Konovalova, I., Paliutina, G., Anisimov, A., 2012. Light-Emitting Diodes: on the Way to Combinatprial Lightong Technologies for Basic Research and Crop Production. Acta Hortic. 956, 171-174.

Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G., 2004. Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. New Phytologist, 163, 547-561.

Tsormpatsidis, E., Henbest, R.G., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, A., 2008. UV irradiance as a major influence on growth, development and secondary product of commercial importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. Environ. Exp. Bot. 63, 232-239.

Winkel-Shirley, B., 2002. Biosinthesys of flavonoids and effects of stress. Curr. Opin. Plant Biol. 5, 218-223.

Yoshida, H., Hikosaka, S., Goto, E., 2012. Effects of Light Quality and Light Period on Flowering of Everbearing Strawberry in a Closed Plant Production System. Acta Hortic. 956, 107-113.

Zhang, H.H., Yang, Q., Yang, H.J., 2011. Self-adaptive and precise supplementary lighting system for plant with controllable LED intensity. J. Trans. CSAE 27, 153-158.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.