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

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1 **TITLE: OPTIMAL RED:BLUE RATIO IN LED**
2 **LIGHTING FOR NUTRACEUTICAL INDOOR**
3 **HORTICULTURE.**

4
5 **RUNNING TITLE: LED SPECTRA FOR**
6 **NUTRACEUTICAL PLANTS**

7
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20
21 **ABSTRACT**

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

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

34

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

37

38 **1. INTRODUCTION**

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

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

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

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

88

89 **2. MATERIALS AND METHODS**

90 *2.1. Plant Material and Growth Conditions*

91 Four experiments were consecutively conducted in a 9 m² walk-in growth chamber at the Department
92 of Agricultural Sciences (DipSA) of the University of Bologna, Italy. Fifteen days old plantlets of
93 basil (*exp. 1# and 2#*) and strawberry (*exp. 3# and 4#*) were transplanted into plastic 1 liter-pots (1
94 plant per pot) filled with a volcanic growing media (particle size 0-15mm; pH 7; humidity 18.8%; EC
95 0.23 mS cm⁻¹; total organic carbon 2.2% DM; N 0.42% DM; P 0.12 % DM; K⁺ 3.7 % DM; Ca²⁺ 2.5
96 % DM; Mg²⁺ 1.2 % DM; Fe²⁺ 2.6 % DM). Plants were automatically drip irrigated three times per
97 day to ensure adequate substrate moisture. Planting density was respectively 24 and 9 plants m⁻², for
98 basil and strawberry. Fertigation was carried out once a week by adding to the irrigation water at the
99 following concentrations: N-NO₃⁻ : 6.0 mM; N-NH₄⁺:1.0 mM; PO₄³⁻ : 3.0 mM; K⁺: 4.0 mM; SO₄²⁻ :
100 7.0 mM; Ca²⁺ : 5.0 mM; Mg²⁺ : 4.0 mM; microelements in traces. The growth chamber was
101 automatically regulated at 21±2°C, 55-70% of humidity and 450 ppm CO₂. The experiments were

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

104

105 *2.2 Treatments and Experimental Design*

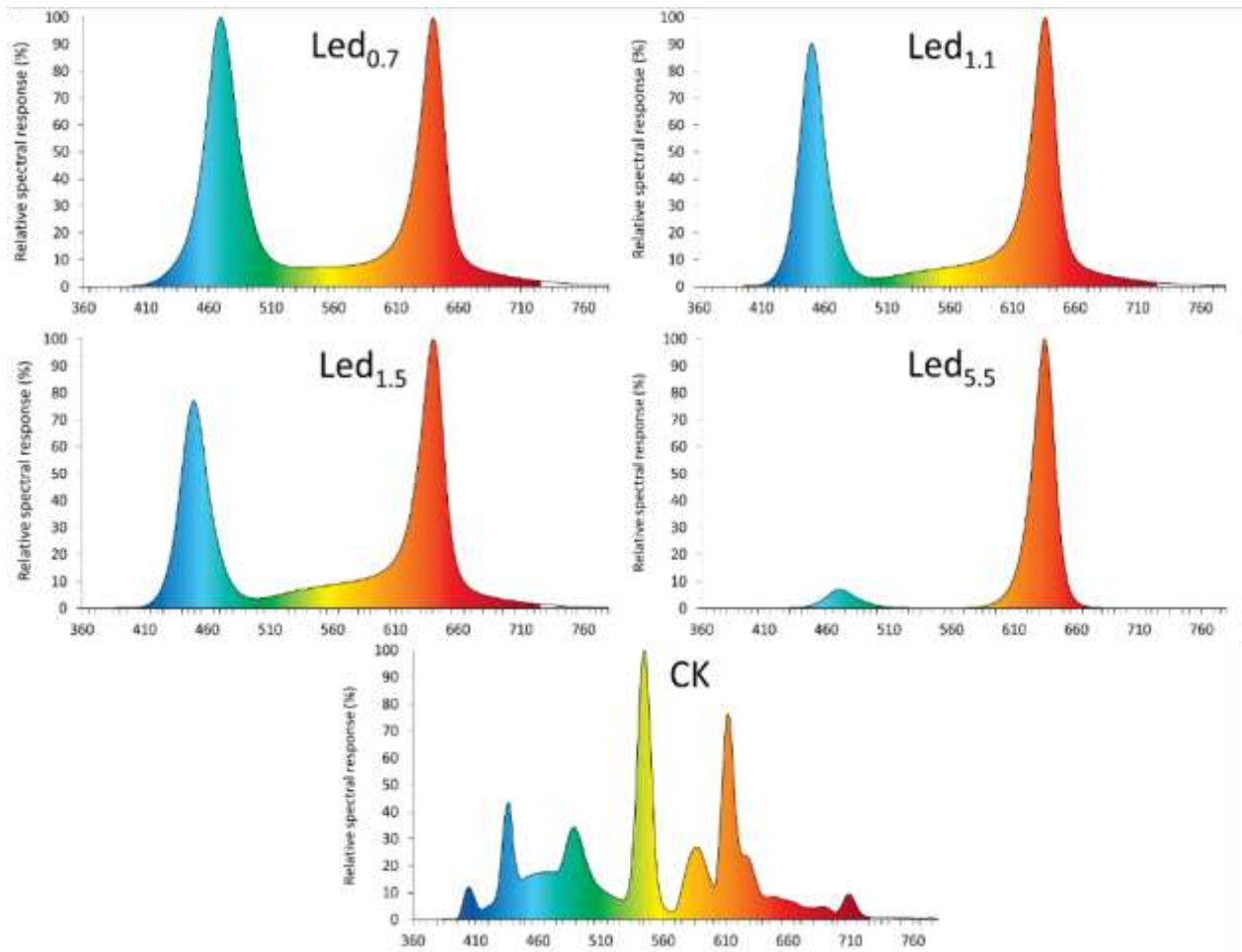
106 Different artificial light treatments (16/8 h light/dark) were applied by dividing the growth chamber
 107 into separate sectors (on shelves fixed on the chamber walls and sealed using white lightproof sealed
 108 walls, each 0.3 m²) and placing lamps with different spectrum and same photosynthetic photon flux
 109 density (PPFD) over the canopy (measured on top leaves, lamps at distance of 10 cm) in measure of
 110 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Five treatments (each of them replicated in four sectors, on 1.2 m²), derived from
 111 the combination of 4 different LED lamps (Led_{0.7}, Led_{1.1}, Led_{1.5}, Led_{5.5}) and a fluorescent light (CK
 112 as a control) (TL-D 90 De Luxe 58W 950, Philips, Amsterdam, The Netherlands), were compared
 113 (see specs in **Table 1** and **Fig. 1**). Ratio between red and blue portions of the spectrum were calculated
 114 by defining the surfaces of the whole spectrum within the red (635-700 nm) and the blue (450-490
 115 nm) wavelengths. One LED lamp was used in each 0.3 m² sector, kindly provided by Bulbo
 116 (Bologna, Italy, further specifics on www.bulbolight.com).

117

118 **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

119



120

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

122

123 *2.3 Vegetative and physiological measurements*

124 At harvest time, total plant fresh weight (FW) was measured in all experiments and total fruit yield
 125 determined in strawberry. Additionally, basil leaves and strawberry fruits were immersed in liquid
 126 nitrogen and kept at -80 °C for biochemical analysis. Measurements of leaf gas exchanges were
 127 performed for both plant species on attached leaf samples using a CIRAS-2 (PPSystem, Hitchin, UK)
 128 infrared gas analyser (closed system) with a Parkinson's Automatic Universal Leaf Cuvette (air inside
 129 the cuvette with the following characteristics: 26°C, CO₂ 450 ppm and 300 cm³ min⁻¹ flow rate)
 130 equipped with 18-mm diameter, 2.5-cm² area cuvette inserts. Net photosynthesis (*A*) was measured
 131 at 14 DAT on four completely unfolded leaves per each plant species and treatment. Energy use

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

134

135 *2.4 Biochemical determination*

136 *2.4.1 Extraction of phenolic compound*

137 5g of basil leaf and strawberry fruit frozen samples were thawed at room temperature and
138 homogenized with 20mL of methanol/H₂O/acetone (60:30:10 v/v/v) (Hartmann et al., 2008). Each
139 mixture was centrifuged (ALLEGRA™ 25R Centrifuge, BECKMAN, El Cajon, CA, USA) at 15.300
140 xg for 10 minutes and the supernatant was collected. The extraction was repeated one time and the
141 final extract (10 mL) was used for the determination of total phenolic and flavonoid content and total
142 antioxidant capacity.

143

144 *2.4.2 Determination of total phenolic and flavonoid contents*

145 Total phenolic content (TPC) was determined according to Folin-Ciocalteu colorimetry method
146 (Giusti and Wrolstad, 2002). Briefly, 100 μL of extracted sample was mixed with 1.5 mL of distilled
147 water in 2.5 mL plastic cuvette followed by 100 μL of Folin-Ciocalteu phenolic reagent and
148 incubated for 5 minutes at room temperature. After mixing, 300 μL of 20% sodium carbonate were
149 added and mixed thoroughly then incubated for 2 h at room temperature. The sample absorbance was
150 measured at 765 nm by spectrophotometer (DU530® life science UV/VIS spectrophotometer,
151 BECKMAN, El Cajon, CA, USA). All samples were measured in duplicate and the total phenolic
152 content was expressed as gallic acid equivalent in milligram per g of fresh weight of basil leaves and
153 strawberry fruits. Total flavonoid content (TFC) was determined by aluminium chloride colorimetric
154 assay (Zhishen et al.,1999). Briefly, 750 μL of extracted sample was mixed with 750 μL of distilled
155 water, and 75 μL of 5% NaNO_2 was added in a 2.5 mL plastic cuvette. After 6 minutes, 150 μL of
156 10% AlCl_3 was added, followed by 500 μL of 1 M NaOH after 5 minutes. Then the sample absorbance
157 was measured at 510 nm by spectrophotometer and the calibration was carried out by a standard curve

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

160

161 *2.4.3 Total Antioxidant Capacity*

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

164 The composition of FRAP reagents was:

- 165 1. Acetate buffer 300 mM pH 3.6: 3.1 g sodium acetate tri-hydrate were added with 16 mL of
166 glacial acetic acid and filled to the volume to 1 L with distilled water.
- 167 2. TPTZ (2, 4, 6-tripyridyl-s-triazine) 10 mM in 40 mM HCl
- 168 3. FeCl₃.6H₂O 20 mM

169 The final FRAP reagent was prepared by mixing 1, 2 and 3 in the ratio of 10:1:1 at the time of use
170 and covered with aluminium. Briefly, freshly prepared FRAP reagent (2.4 mL) was mixed with 80
171 µL of sample (0.1 g mL⁻¹) in duplicate. The mixture was equilibrated for 1 hour at room temperature
172 before absorbance was measured at 593 nm. Aqueous solutions of Fe-(II) (FeSO₄.6H₂O) in the
173 concentration range of 125-1250 µM were used for calibration of the FRAP assay. FRAP values were
174 expressed as mmol Fe²⁺ kg⁻¹ FW.

175

176 *2.4.4 Nitrates content determination*

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

184

185 *2.5 Statistical Analysis*

186 The study employed a randomized block design with single experiments as elemental block and
187 randomised design within the block with four replications and each replication represented by 8 and
188 3 plants (basil and strawberry, respectively). Data were analysed by two-ways ANOVA and the
189 means were compared by Least Significance Difference (LSD), at 5% significance level.

190

191 **3. RESULTS**192 *3.1 Fresh Weight and Energy Use Efficiency Responses*

193 In basil, leaf fresh weight was significantly lower in LED treatments having a red:blue ratio of 1.1,
194 1.5 and 5.5 compared to the CK, with the exception of Led_{0.7} having a similar leaf biomass (**Table**
195 **2**). It was observed that decreasing the blue spectrum intensity, the basil fresh weight decreased
196 almost constantly: while red:blue ratio increased by about 1.5, 2.0 and 8.0 folds, plant FW was
197 diminished by 16%, 39% and 68% referring to the highest plant FW under the light Led_{0.7} (**Fig. 2**).
198 Consistently, greatest EUE performances were observed in plants grown under Led_{0.7} resulting in a
199 5-fold increase in the biomass produced per kW consumed as compared to CK (**Table 2**).

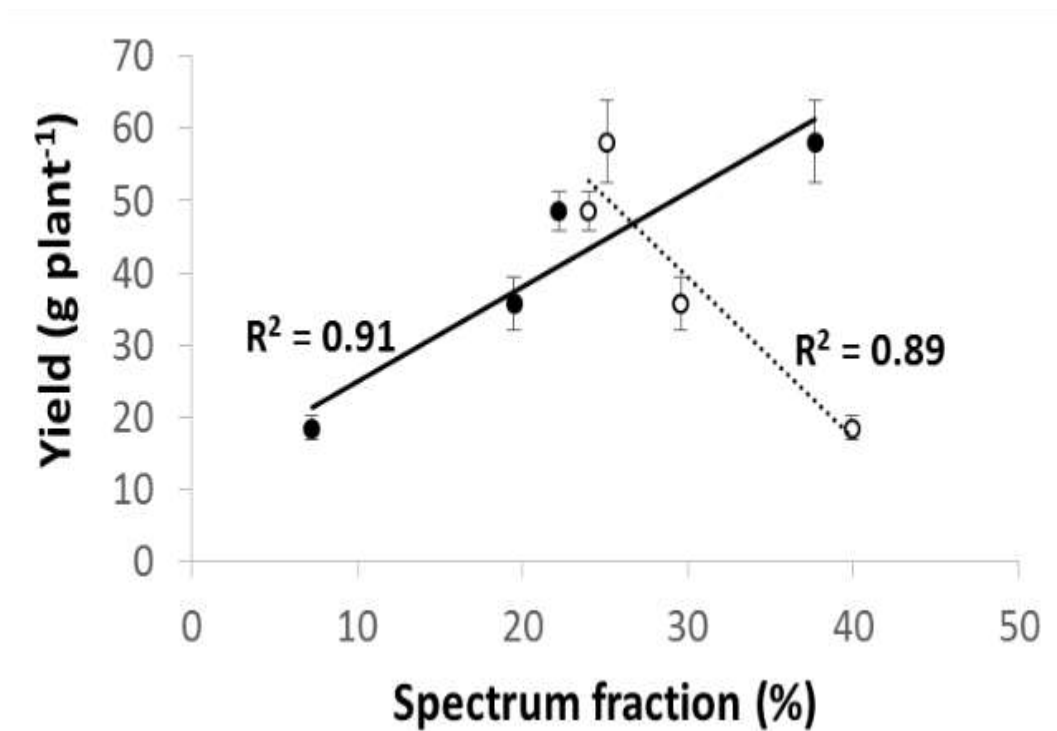
200 In strawberry, higher plant fresh weight was observed in all LED treatments, resulting in a biomass
201 increase up to 20 g plant⁻¹ as compared to CK (**Table 2**). EUE was enhanced in all LED treatments,
202 being significantly different from CK (**Table 2**). The greatest strawberry fruit production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

208

209 **Table 2.** Foliar and fruit biomass (FW, g plant⁻¹) and Energy Use Efficiency (EUE, g kW⁻¹) in plants
 210 of basil (*Ocimum basilicum* L.) and strawberry (*Fragaria x Ananassa*) as affected by light (see specs
 211 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}	<u>58.1</u>	a	<u>83.0</u>	a	25.4	b	<u>36.2</u>	a	<u>19.0</u>	a	<u>27.1</u>	a
Led _{1.1}	48.6	b	69.4	b	<u>27.9</u>	ab	<u>39.9</u>	a	<u>23.0</u>	a	<u>32.9</u>	a
Led _{1.5}	35.7	c	44.6	c	<u>35.8</u>	a	<u>44.8</u>	a	<u>16.5</u>	ab	<u>20.6</u>	ab
Led _{5.5}	18.5	d	23.2	d	24.7	b	<u>35.0</u>	a	5.3	b	6.6	b
CK	<u>50.9</u>	a	17.6	d	15.8	c	5.5	b	7.8	b	2.7	b

212



213

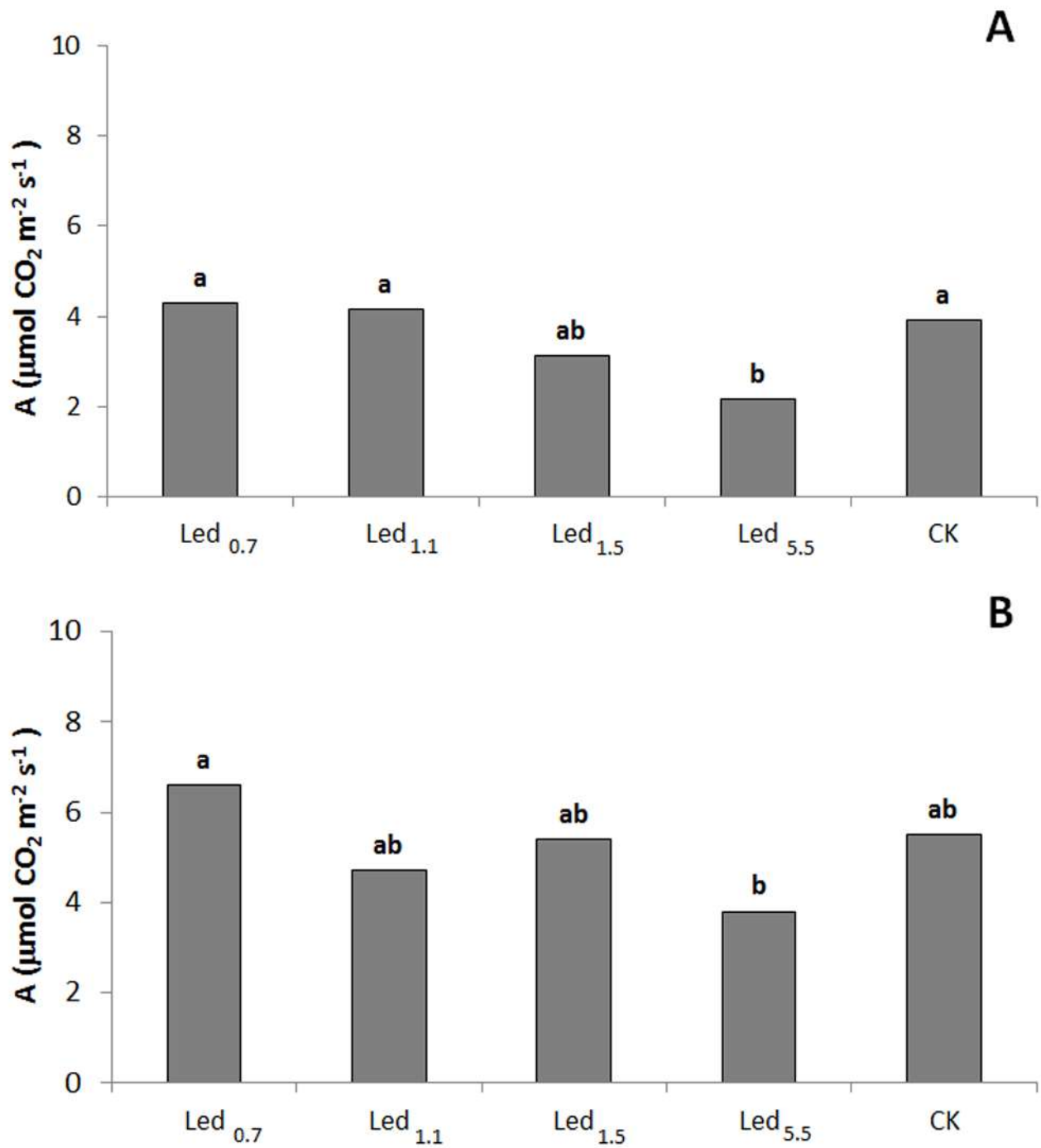
214 **Figure 2.** Linear relationship between yield (g plant⁻¹) and the spectral fraction of blue (closed
 215 circles) and red (open circles) in basil (*Ocimum basilicum* L.). Mean values ± SE, n=24.

216

217 *3.2 Assimilation Rates*

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

222



224

225 **Figure 3.** Assimilation rate (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in plants of basil (*Ocimum basilicum* L., **A**) and
 226 strawberry (*Fragaria x Ananassa*, **B**) as affected by light (see specs in table 1). Mean values. Different
 227 letters indicate significant differences at $P \leq 0.05$.

228

229 *3.4 Antioxidant Capacity, Polyphenols and Flavonoids*

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

236

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

240

Light treatment	Basil								Strawberry							
	Leaves				Fruits				Leaves							
	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}	<u>4.2</u>	<u>a</u>	<u>12.5</u>	<u>a</u>	<u>3.5</u>	<u>a</u>	658	b	4.3	b	1.4	a	0.31	b	996	b
Led _{1.1}	<u>4.3</u>	<u>a</u>	<u>12.3</u>	<u>a</u>	<u>3.6</u>	<u>a</u>	528	b	<u>4.4</u>	<u>ab</u>	1.3	a	0.32	b	<u>1237</u>	<u>a</u>
Led _{1.5}	<u>4.2</u>	<u>a</u>	<u>12.4</u>	<u>a</u>	<u>3.4</u>	<u>a</u>	718	b	<u>4.5</u>	<u>a</u>	1.4	a	0.32	b	1094	b
Led _{5.5}	<u>4.1</u>	<u>a</u>	<u>11.6</u>	<u>a</u>	<u>3.2</u>	<u>a</u>	<u>984</u>	<u>a</u>	<u>4.5</u>	<u>a</u>	1.4	a	0.32	b	949	b
CK	3.6	b	8.9	b	2.1	b	<u>1046</u>	<u>a</u>	<u>4.5</u>	<u>a</u>	1.3	a	<u>0.34</u>	<u>a</u>	<u>1276</u>	<u>a</u>

241

242 3.5 Nitrates Content

243 Highest nitrates contents in basil leaves were associated with CK and Led_{5.5} (mean value 1015 mg
 244 kg⁻¹ FW) treatments (**Table 3**). Nitrates content was significantly lower in basil plants grown under
 245 Led_{0.7}, Led_{1.1} and Led_{1.5} (mean value 635 mg kg⁻¹ FW). As regard strawberry leaves, lower values
 246 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
 247 CK (mean value 1256 mg kg⁻¹ FW) (**Table 3**).

248

249 **4. DISCUSSION**250 *4.1 LED influence on plant growth and yield*

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

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

267

268 *4.2 How do plants respond to different spectral compositions?*

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

274 enhanced from 9 to 18 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Nevertheless, strawberry fresh weight was further improved
275 (**Table 2**) under Led_{1.1} and Led_{1.5}, confirming that a species-specific mixture of red and blue spectral
276 components is necessary for proper plant development (Samuolienė et al., 2010). The reduction of
277 fruit yield in plants grown under red LED light (Led_{5.5}) or CK (**Table 2**) may be associated with
278 reduced flowering and fruiting as a consequence of insufficient blue light fraction (Yoshida et al.,
279 2012).

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

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

293 It is widely known that red spectral regions of light have the strongest impact on the rates of
294 photosynthesis in plants. Photosystems (PS) I and II absorb wavelengths around 650 nm (PSII) and
295 700 nm (PSI) (Schopfer and Brennicke, 2010). In the present study however, all species showed better
296 photosynthetic performance when an additional proportion of blue light was present, namely under
297 Led_{0.7}, Led_{1.1}, Led_{1.5} and CK (**Fig. 3**). However, given the lower yield of photosynthesis under blue
298 monochromatic light, it is still suggested to use a combination of red and blue spectral regions
299 (Domurath et al., 2012). According to these results overall, the photosynthetic performances did not

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

306

307 *4.3 Optimal spectral compositions lead to improved nutraceutical properties of plant products*

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

322 The use of LED lights for indoor cultivation of arugula, has been shown to cause an overall increase
323 (+22%) in the flavonoids concentration as compared to HPS lighting. However, LED did not
324 positively affect strawberry plant in terms of antioxidant compounds. Overall, significantly higher

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

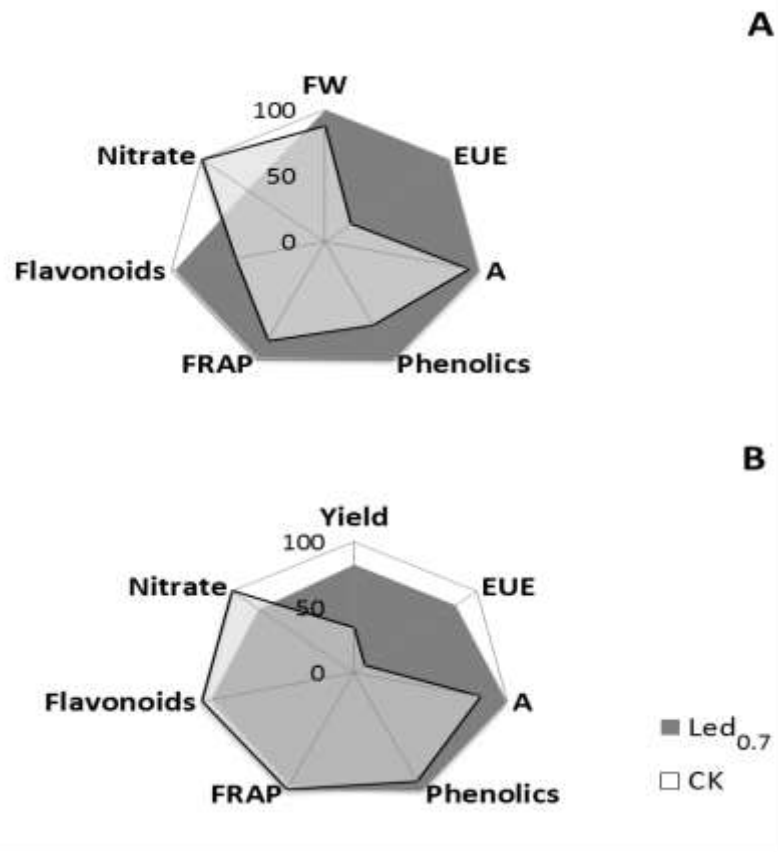
327 Consistent with the increase in antioxidants, a general decrease in the NO_3^- concentration in basil
328 green tissue was associated with LED as compared to CK. In plants, nitrate accumulation is
329 counteracted by the activity of nitrate reductase, an enzyme regulated by nitrate availability
330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

339

340 **5. CONCLUSIONS**

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

348



349

350 **Figure 4.** Representation summarizing the various parameters that have been recorded in this study.

351 Best performant LED light (Led_{0.7}) is compared for each species with the relative fluorescent control

352 (CK). Relative performances (as compared to maximum detected value) in plants of basil (*Ocimum*

353 *basilicum* L., **A**) and strawberry (*Fragaria* x *Ananassa*, **B**) as affected by light (see specs in Table 1).

354

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360 **7. REFERENCES**

- 361 Aaby, K., Wrolstad, R.E., Ekeberg, D., Skrede, G., 2007. Polyphenol composition and antioxidant
362 activity in strawberry purees; impact of achene level and storage. *J. Agric. Food Chem* 55, 5156-
363 5166.
- 364 Abiusi, F., Sampietro, G., Marturano, G., Biondi, N., Rodolfi, L., D'Ottavio, M., Tredici, M.R., 2013.
365 Growth, photosynthetic efficiency, and biochemical composition of *Tetraselmis suecica* F&M-
366 M33 grown with LEDs of different colors. *Biotechnol Bioeng* 111, 956-964.
- 367 Becker, T.W., Caboche, M., Carrayol, E., Hirel, B., 1992. Nucleotide sequence of tobacco cDNA
368 encoding plastidic glutamine synthetase and light inducibility, organ specificity and diurnal
369 rhythmicity in the expression of the corresponding genes of tobacco and tomato. *Plant Mol. Biol.*
370 19, 367-379.
- 371 Benzie, F.F., Strain, J.J., 1999. Ferric Reducing/Antioxidant Power assay: Direct Measure of Total
372 antioxidant Activity of Biological Fluids and Modified Version for Simultaneous Measurement
373 of Total Antioxidant Power and Ascorbic Acid Concentration. *Meth. Enzymol.* 299,15-23
- 374 Crawford, N.M., 1995. Nitrate: Nutrient and Signal for Plant Growth. *Plant Cell* 7, 859–868.
- 375 Domurath, N., Schroeder, F-G., Glatzel, S., 2012. Light Response Curve of Selected Plants under
376 Different Light Conditions. *Acta Hortic.* 956, 291-298.
- 377 Giliberto, L., Perrota, G., Pallara, P., Weller, J.L., Fraser, P.D., Bramley, P.M., Fiore, A., Tavazza,
378 M., Giuliano, G., 2005. Manipulation of blue light photoreceptor cryptochrome 2 in tomato
379 affects vegetative development, flowering time, and fruit antioxidant content. *Plant Physiol.* 137,
380 199-208.
- 381 Giusti, M., Wrolstad, R.E., 2002. Determination of Total Phenolics. *Curr. Protoc. Food Analyt.*
382 Chem. II.1.1-11.1.8.
- 383 Goto, E., 2012. Plant production in a closed plant factory with artificial lighting. *Acta Hortic.* 956,
384 37-49
- 385 Hartmann, A., Patz, C.D., Andlauer, W., Dietrich, H., Ludwig, M., 2008. Influence of processing on
386 quality parameters of strawberries. *J. Agric. Food Chem.* 56, 9484-9489.
- 387 Hernández, R., Kubota, C., 2012. Tomato seedling growth and morphological responses to
388 supplemental LED lighting red:blue ratios under varied daily solar light integrals. *Acta Hortic.*
389 956, 187-194.
- 390 Hogewoning, S.W., Trouwborst, G., Meinen, E., van Ieperen, W., 2012. Finding the optimal growth-
391 light spectrum for greenhouse crops. *Acta Hortic.* 956, 357-363.
- 392 Horton, P., 2000. Prospects for crop improvement through the genetic manipulation of
393 photosynthesis: morphological and biochemical aspects of light capture. *J. Exp. Bot.* 51, 475-485.

- 394 Hunt, J.E., McNeil, D.L., 1998. Nitrogen status affects UV-B sensitivity of cucumber. *Aust. J. Plant*
395 *Physiol.* 25, 79-86.
- 396 Kage, H., Alt, C., Stutzel, H., 2002. Nitrogen concentration of cauliflower organs as determined by
397 organ size, N supply, and radiation environment. *Plant Soil* 246, 201-209.
- 398 Kataoka, I., Sugiyama, A., Beppu, K., 2003. Role of ultraviolet radiation in accumulation of
399 anthocyanin in berries of 'Gros Colman' grapes (*Vitis Vinifera* L.). *J. JPN. Soc. Hortic. Sci* 72, 1-
400 6.
- 401 Kopsell, D.A., Kopsell, D.E., 2008. Genetic and environmental factors affecting plant
402 lutein/zeaxanthin. *Agro Food Ind. Hi. Tech.* 19, 44-46.
- 403 Koyama, K., Ikeda, H., Poudel, P.R., Goto-Yamamoto, N., 2012. Light quality affects flavonoid
404 biosynthesis in young berries of Cabernet Sauvignon grape. *Phytochem.*, 78, 54-64.
- 405 Li, Q., Kubota, C., 2009. Effects of supplemental light quality on growth and phytochemicals of baby
406 leaf lettuce. *Environ. Exp. Bot.* 67, 59-64.
- 407 Milner, J., 2002. Functional foods and health: A US perspective. *Br. J. Nutr.* 88, S151-S158.
- 408 Norling, C.L., Wiggins, H.N., Crawford, J.I., Wotton, A.W.M., 2012. Flexible spectra LED arrays
409 for sole source lighting and growth comparisons with conventional high pressure discharge
410 lighting using *Arabidopsis thaliana*. *Acta Hortic.* 956, 113-120.
- 411 Ohashi-Kaneko, K., Takase, M., Kon, N., Fujiwara, K., 2007. Effect of light quality on growth and
412 vegetable quality in leaf lettuce, spinach and komatsuna. *Environ. Control Biol.* 45, 189-198.
- 413 Orsini, F., De Pascale, S., 2007. Daily variation in leaf nitrate content of two cultivars of
414 hydroponically grown basil. *Acta Hortic.* 747, 203-210.
- 415 Sah, R.N., 1994. Nitrate-nitrogen determination: a critical review. *Commun Soil Sci Plant Anal.* 25,
416 2841-2869.
- 417 Samuolienė, G., Brazaitytė, A., Urbonaviciūtė, A., Sabajevienė, G., Duchovskis, P., 2010. The effect
418 of red and blue light component on the growth and development of frigo strawberries.
419 *Zemdirbyste-Agric.* 97, 99-104.
- 420 Samuolienė, G., Brazaitytė, A., Sirtautas, R., Sakalauskiene, S., Jankauskiene, J., Duchovskis, P.,
421 2012. The impact of supplementary short-term Red LED lighting on the antioxidant properties of
422 microgreens. *Acta Hortic.* 956, 649-655.
- 423 Sase, S., Mito, C., Okushima, L., 2012. Effect of Overnight Supplemental Lighting with Different
424 Spectral LEDs on the Growth of Some Leafy Vegetables. *Acta Hortic.* 956, 327-333.
- 425 Schamp, B., Pauwels, E. and Gobin, B., 2012. Developing led light recipes for multi-layering
426 systems: LED as an alternative for HPS in forcing of rhododendron. *Acta Hortic.* 956: 121-128

- 427 Schopfer, P., Brennicke, A., 2010. Pflanzenphysiologie. Spektrum Akademischer Verlag,
428 Heidelberg. Seventh edition.
- 429 Speijers, G.J.A., 1996. Nitrite (and potential endogenous formation of N-nitroso compounds). In:
430 Toxicological evaluation of certain food additives and contaminants in food, WHO Food
431 Additives Series, Ginevra 35, 269-323.
- 432 Tarakanov, I., Yakovleva, O., Konovalova, I., Paliutina, G., Anisimov, A., 2012. Light-Emitting
433 Diodes: on the Way to Combinatorial Light Technologies for Basic Research and Crop
434 Production. Acta Hort. 956, 171-174.
- 435 Tattini, M., Galardi, C., Pinelli, P., Massai, R., Remorini, D., Agati, G., 2004. Differential
436 accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess
437 light and drought stress. New Phytologist, 163, 547-561.
- 438 Tsormpatsidis, E., Henbest, R.G., Davis, F.J., Battey, N.H., Hadley, P., Wagstaffe, A., 2008. UV
439 irradiance as a major influence on growth, development and secondary product of commercial
440 importance in Lollo Rosso lettuce 'Revolution' grown under polyethylene films. Environ. Exp.
441 Bot. 63, 232-239.
- 442 Winkel-Shirley, B., 2002. Biosynthesis of flavonoids and effects of stress. Curr. Opin. Plant Biol. 5,
443 218-223.
- 444 Yoshida, H., Hikosaka, S., Goto, E., 2012. Effects of Light Quality and Light Period on Flowering of
445 Everbearing Strawberry in a Closed Plant Production System. Acta Hort. 956, 107-113.
- 446 Zhang, H.H., Yang, Q., Yang, H.J., 2011. Self-adaptive and precise supplementary lighting system
447 for plant with controllable LED intensity. J. Trans. CSAE 27, 153-158.
- 448 Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents in mulberry
449 and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559.
- 450