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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Piovene, C., Orsini, F., Bosi, S., Sanoubar, R., Bregola, V., Dinelli, G., et al. (2015). Optimal red: Blue ratio in led lighting for nutraceutical indoor horticulture. *SCIENTIA HORTICULTURAE*, 193, 202-208 [10.1016/j.scienta.2015.07.015].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/517483> since: 2015-10-28

*Published:*

DOI: <http://doi.org/10.1016/j.scienta.2015.07.015>

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(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

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*Optimal red:blue ratio in led lighting for nutraceutical indoor horticulture*

which has been published in final form in *Scientia Horticulturae*

Volume 193, 22 September 2015, Pages 202-208

The final published version is available online at:

<https://doi.org/10.1016/j.scienta.2015.07.015>

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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  $\text{NO}_3^-$  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 m<sup>2</sup> 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<sup>-1</sup>; total organic carbon 2.2% DM; N 0.42% DM; P 0.12 % DM; K<sup>+</sup> 3.7 % DM; Ca<sup>2+</sup> 2.5  
96 % DM; Mg<sup>2+</sup> 1.2 % DM; Fe<sup>2+</sup> 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<sup>-2</sup>, for  
98 basil and strawberry. Fertigation was carried out once a week by adding to the irrigation water at the  
99 following concentrations: N-NO<sub>3</sub><sup>-</sup> : 6.0 mM; N-NH<sub>4</sub><sup>+</sup>:1.0 mM; PO<sub>4</sub><sup>3-</sup> : 3.0 mM; K<sup>+</sup>: 4.0 mM; SO<sub>4</sub><sup>2-</sup> :  
100 7.0 mM; Ca<sup>2+</sup> : 5.0 mM; Mg<sup>2+</sup> : 4.0 mM; microelements in traces. The growth chamber was  
101 automatically regulated at 21±2°C, 55-70% of humidity and 450 ppm CO<sub>2</sub>. 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*

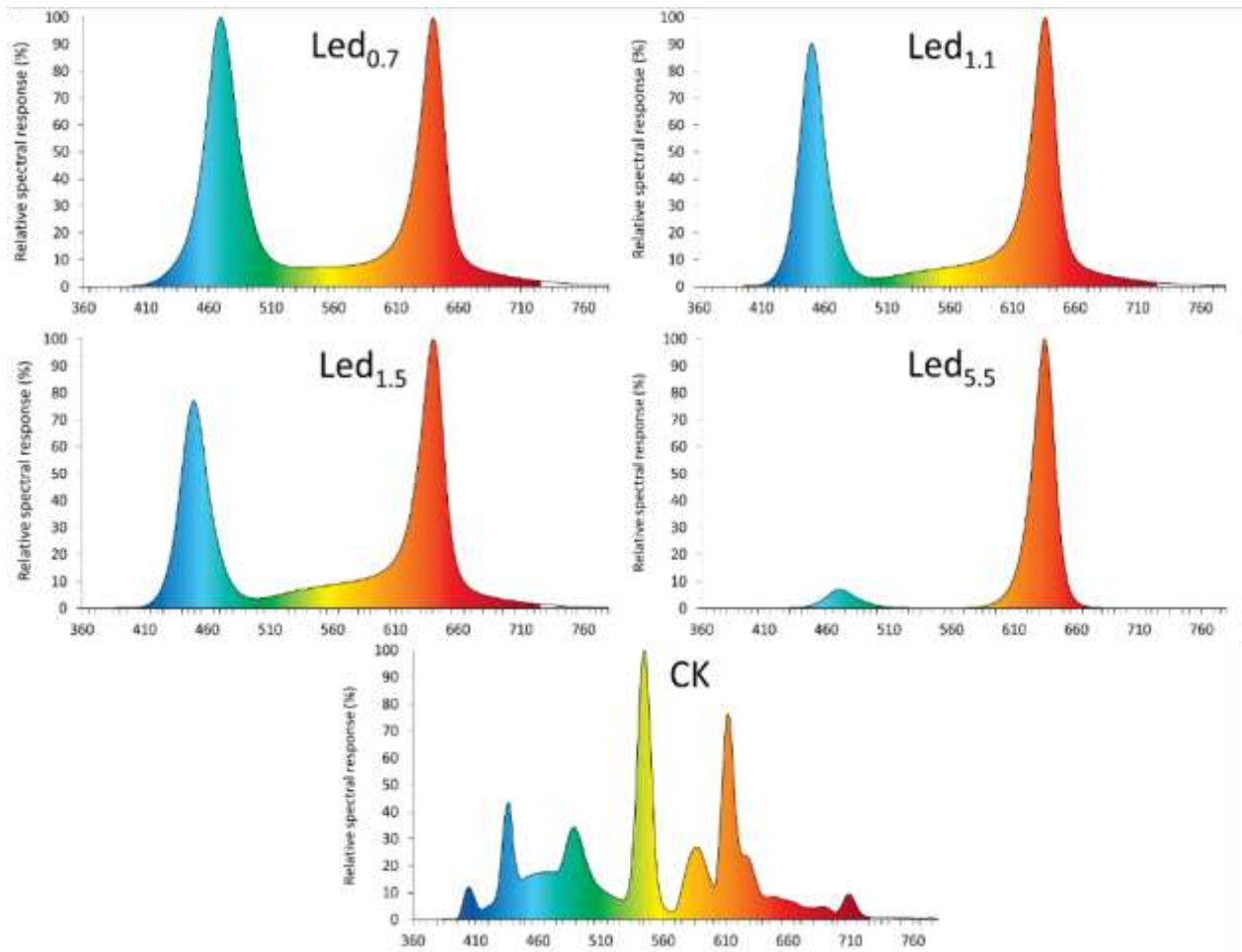
106 Different artificial light treatments (16/8 h light/dark) were applied by dividing the growth chamber  
 107 into separate sectors (on shelves fixed on the chamber walls and sealed using white lightproof sealed  
 108 walls, each 0.3 m<sup>2</sup>) and placing lamps with different spectrum and same 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 m<sup>2</sup>), derived from  
 111 the combination of 4 different LED lamps (Led<sub>0.7</sub>, Led<sub>1.1</sub>, Led<sub>1.5</sub>, Led<sub>5.5</sub>) 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 m<sup>2</sup> sector, kindly provided by Bulbo  
 116 (Bologna, Italy, further specifics on [www.bulbolight.com](http://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 <sup>-1</sup> h <sup>-1</sup>	Red (%)	Blue (%)	Red:Blue ratio	
40	40	20	1.5	25.1	37.7	0.7	Led <sub>0.7</sub>
40	30	30	1.5	24.0	22.2	1.1	Led <sub>1.1</sub>
50	20	30	1.5	29.6	19.5	1.5	Led <sub>1.5</sub>
90	10	0	1.5	39.9	7.3	5.5	Led <sub>5.5</sub>
-	-	-	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, CO<sub>2</sub> 450 ppm and 300 cm<sup>3</sup> min<sup>-1</sup> flow rate)  
 130 equipped with 18-mm diameter, 2.5-cm<sup>2</sup> 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  $\text{g kW}^{-1}$ .

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/H<sub>2</sub>O/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  $\mu\text{L}$  of extracted sample was mixed with 1.5 mL of distilled  
147 water in 2.5 mL plastic cuvette followed by 100  $\mu\text{L}$  of Folin-Ciocalteu phenolic reagent and  
148 incubated for 5 minutes at room temperature. After mixing, 300  $\mu\text{L}$  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  $\mu\text{L}$  of extracted sample was mixed with 750  $\mu\text{L}$  of distilled  
155 water, and 75  $\mu\text{L}$  of 5%  $\text{NaNO}_2$  was added in a 2.5 mL plastic cuvette. After 6 minutes, 150  $\mu\text{L}$  of  
156 10%  $\text{AlCl}_3$  was added, followed by 500  $\mu\text{L}$  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. FeCl<sub>3</sub>.6H<sub>2</sub>O 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<sup>-1</sup>) 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) (FeSO<sub>4</sub>.6H<sub>2</sub>O) in the  
173 concentration range of 125-1250 µM were used for calibration of the FRAP assay. FRAP values were  
174 expressed as mmol Fe<sup>2+</sup> kg<sup>-1</sup> 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<sup>-1</sup> 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<sub>0.7</sub> 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<sub>0.7</sub> (**Fig. 2**).  
198 Consistently, greatest EUE performances were observed in plants grown under Led<sub>0.7</sub> resulting in a  
199 5-fold increase in the biomass produced per kW consumed as compared to CK (**Table 2**).

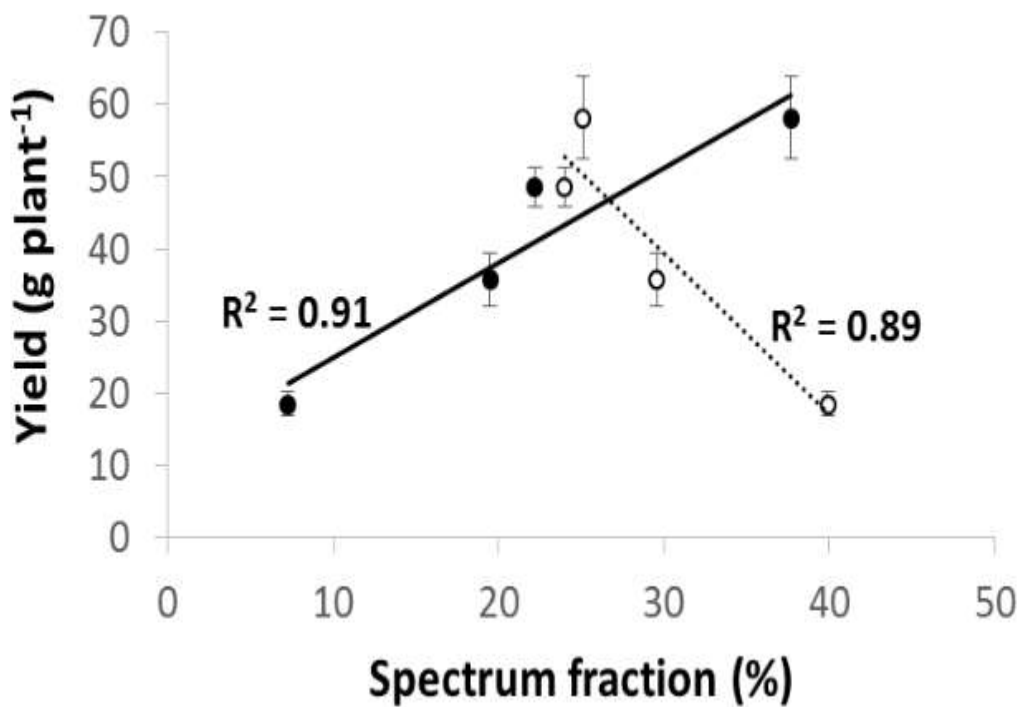
200 In strawberry, higher plant fresh weight was observed in all LED treatments, resulting in a biomass  
201 increase up to 20 g plant<sup>-1</sup> as compared to CK (**Table 2**). EUE was enhanced in all LED treatments,  
202 being significantly different from CK (**Table 2**). The greatest strawberry fruit production (g fruit  
203 plant<sup>-1</sup>) was observed in Led<sub>0.7</sub> and Led<sub>1.1</sub> treatments (**Table 2**), both achieving more than a 3-fold  
204 higher productivity as compared to CK and Led<sub>5.5</sub> 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<sup>-1</sup>  
207 (Led<sub>0.7</sub>, Led<sub>1.1</sub> and Led<sub>1.5</sub> vs 4.6 g kw<sup>-1</sup>) (Led<sub>5.5</sub> and CK) (**Table 2**).

208

209 **Table 2.** Foliar and fruit biomass (FW, g plant<sup>-1</sup>) and Energy Use Efficiency (EUE, g kW<sup>-1</sup>) 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 <sup>-1</sup> )		EUE (g kW <sup>-1</sup> )		FW (g plant <sup>-1</sup> )		EUE (g kW <sup>-1</sup> )		FW (g plant <sup>-1</sup> )		EUE (g kW <sup>-1</sup> )	
Led <sub>0.7</sub>	<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 <sub>1.1</sub>	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 <sub>1.5</sub>	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 <sub>5.5</sub>	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

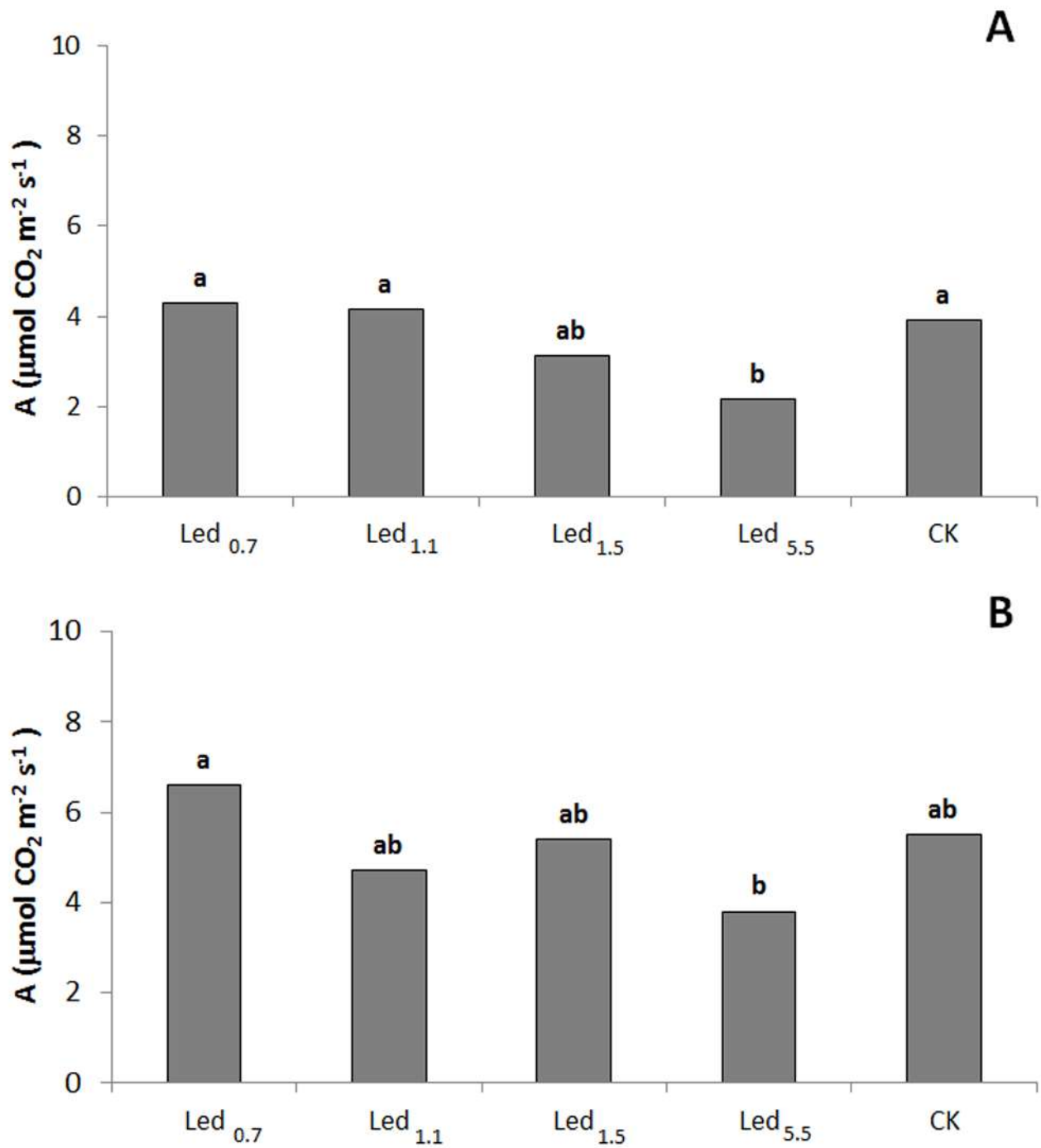
214 **Figure 2.** Linear relationship between yield (g plant<sup>-1</sup>) and the spectral fraction of blue (closed  
 215 circles) and red (open circles) in basil (*Ocimum basilicum* L.). Mean values ± SE, n=24.

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<sub>5.5</sub> 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<sub>5.5</sub> compared to Led<sub>0.7</sub> (**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<sub>0.7</sub> (-4% compared to Led<sub>1.5</sub>, Led<sub>5.5</sub> 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 <sup>2+</sup> kg <sup>-1</sup> FW)		TPC (GA g <sup>-1</sup> FW)		TFC (mg CE g <sup>-1</sup> FW)		NO <sub>3</sub> (mg kg <sup>-1</sup> FW)		FRAP (mmol Fe <sup>2+</sup> kg <sup>-1</sup> FW)		TPC (GA g <sup>-1</sup> FW)		TFC (mg CE g <sup>-1</sup> FW)		NO <sub>3</sub> (mg kg <sup>-1</sup> FW)	
Led <sub>0.7</sub>	<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 <sub>1.1</sub>	<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 <sub>1.5</sub>	<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 <sub>5.5</sub>	<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<sub>5.5</sub> (mean value 1015 mg  
 244 kg<sup>-1</sup> FW) treatments (**Table 3**). Nitrates content was significantly lower in basil plants grown under  
 245 Led<sub>0.7</sub>, Led<sub>1.1</sub> and Led<sub>1.5</sub> (mean value 635 mg kg<sup>-1</sup> FW). As regard strawberry leaves, lower values  
 246 were observed in Led<sub>0.7</sub>, Led<sub>1.5</sub> and Led<sub>5.5</sub> (mean value 1013 mg kg<sup>-1</sup> FW) as compared to Led<sub>1.1</sub> and  
 247 CK (mean value 1256 mg kg<sup>-1</sup> 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<sub>0.7</sub>, 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<sub>0.7</sub> (**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<sub>0.7</sub>) (**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<sub>0.7</sub>) (**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<sub>1.1</sub> and Led<sub>1.5</sub>, 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<sub>5.5</sub>) 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<sub>5.5</sub>.  
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<sub>0.7</sub>, Led<sub>1.1</sub>, Led<sub>1.5</sub> 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**).

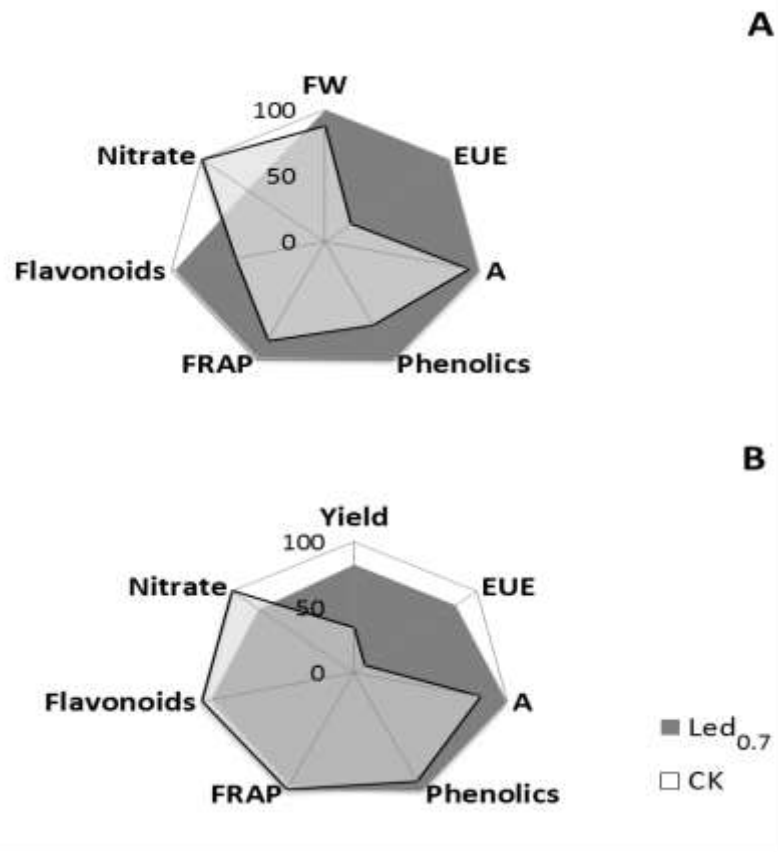
327 Consistent with the increase in antioxidants, a general decrease in the  $\text{NO}_3^-$  concentration in basil  
328 green tissue was associated with LED as compared to CK. In plants, nitrate accumulation is  
329 counteracted by the activity of nitrate reductase, an enzyme regulated by nitrate 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  $\text{NO}_3^-$  concentration in  
336 leaves. In basil plants grown using Led<sub>5.5</sub>, 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<sub>0.7</sub>  
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<sub>0.7</sub>) 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

## 355 6. ACKNOWLEDGEMENTS

356 All LED lights used in the herby presented experiments were prepared by Bulbo, Bologna, Italy. The

357 research was partially funded by the Erasmus+ project Urban Green Train (Urban Green Education

358 for Enterprising Agricultural Innovation). English language was checked by Dr. Monique Centre

359 Stefani.

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