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Effects of Different LED Light Recipes and NPK Fertilizers on Basil Cultivation for Automated and Integrated Horticulture Methods

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Featured Application: The specific application of this work is related to basil cultivation in indoor horticulture and is devoted to the investigation of specific light recipes and fertilizers addition to promote its germination and growth in a controlled environment.

Abstract: This study aims to optimize the conditions for "Genovese" basil (*Ocimum Basilicum*) germination and growth in an indoor environment suitable for horticulture through a synergic effect of light and fertilizers addition. In fact, several studies determined that specific light conditions are capable of enhancing basil growth, but this effect is highly dependent on the environmental conditions. In this study, the effect of different light sources was determined employing a soil with a negligible amount of fertilizer, demonstrating substantial improvement when light-emitting diode (LED) lights (hyper red and deep blue in different combinations) were applied with respect to daylight (Plants height: +30%, Total fresh mass: +50%). Thereafter, a design of experiment approach has been implemented to calculate the specific combination of LED lights and fertilizer useful to optimize the basil growth. A controlled-release fertilizer based on nitrogen, phosphorus, and potassium (NPK) derived from agro-residues was compared with a soil enriched in macronutrients. The results demonstrate significant improvements for the growth parameters with the employment of the controlled-release NPK with respect to enriched soil combined with a ratio of hyper red and deep blue LED light equal to 1:3 (Total fresh mass: +100%, Leaves number: +20%).

Keywords: basil; design of experiments; valorization strategies

1. Introduction

In recent years, the interest toward the applicability of light-emitting diode (LED) lights for indoor cultivation has significantly grown, with the aim of creating a more affordable and ecological production for plants compared to fluorescent lighting and high-pressure sodium (HPS) lamps [1,2]. In fact, artificial light supply presents additional costs compared to cultivation under natural daylight due to energy demand, but it is mandatory in an indoor environment and wherever natural light is not sufficient. For example, these systems are necessary at northern latitudes, where a strong variation of daylight is observed during the year, to address the current challenges that the greenhouse industry is facing (i.e., energy, environment, and market) [3]. Moreover, it is beneficial to increase the product yield and quality of the final product because growing plants in controlled environment allows drastically reducing the use of pesticides. Finally, controlled-environment agriculture is a key subject of the worldwide food optimization system, due to the upcoming population growth and climate changes scenarios [4]. In this context, LED lights are expected to replace conventional light sources due to their longer functional life, lower operating temperatures, and lower energy consumption, thereafter decreasing the operational costs of indoor urban farming. In addition, compared with conventional light sources, LED technology offers an easier and cheap way for light spectrum manipulation, and this is beneficial for crop growth regulation [5,6].

Indeed, LED lights provide tunable wavelengths to be matched to plant photoreceptors, including phytochrome and cryptochrome, in order to have optimal plant growth in terms of morphology (e.g., height, leaf area, thickness, and stem length) and quality (e.g., metabolites) [7–11]. Therefore, this provides a new opportunity to manipulate the quality and quantity of vegetable products for markets and meet the demands of retailers in a future perspective involving artificial intelligence control over a real-time autonomous horticultural system [12]. Artificial intelligence techniques ensure minimal human manipulation by defining very tailored target parameters, ensuring an increased efficiency of the system from the environmental and economic point of view. This vision seeks the full digital control of the light quantity, in order to optimize the crop productivity, which may contribute to an optimized production of food [3,13]. For example, it could be possible not only to control artificial light based on specific natural light conditions but to account for potential physiological changes in the crop [13]. As a result, research is strongly focusing on the application of different LED lights on different plants, and several types of LED-based lamps became available for commercial plant production [14]. Taking into account the more recent research on this topic, generally, blue light is necessary for the morphologically healthy growth of plants mainly involving a transpiration mechanism and stem elongation [15–17]. In contrast, red light plays a major role in plant photosynthesis [18,19]. In fact, it has been demonstrated that plants' chlorophyll A and B efficiently absorb blue and red lights wavelengths, respectively [20]. It has been confirmed that the optimal ratio between blue and red light is of great relevance in determining plant yield, as it has a strong effect on the conversion efficiency of natural photosynthesis, which is peculiar for each vegetable [12,21]. In addition, research on this topic is generally conducted using commercial available light that is not varied during the experimental procedure, and principal results indicate that the complexity of the plant physiology requires a multi-objective optimization approach [12].

In this context, "Genovese" basil (*Ocimum Basilicum*) has been previously studied employing LED lights as the only light source and compared with daylight or fluorescent lamps as control, but no clear trend emerged on the capability for certain light recipes to enhance basil germination or growth. Specifically, the use of red and blue together seems to be preferable; however, the optimal proportions may vary. In fact, depending on several factors, the employment of blue light must be preponderant over red or vice versa, with the possible addition of green or white LED light [22–25]. These variations are mainly due to thedifferent environmental conditions applied during the tests, such as the light surrounding the experiments' area (e.g., into the dark or into a greenhouse) or time of exposure (varying from 21 to 64 days) or daily photoperiod (from 12 to 18 h). In any case, the use of red LED light alone is not recommended, because it leads to a too long time of growth [21]. The different age of collection and analysis of the growth parameters may be one of the other factors leading to the different influence of light. Thus, if the results of some studies lead to the hypothesis that in the first weeks of plant life, blue light is more important than red, other studies refuse it [22]. In addition, in general, the influence of LED lights on the germination phase is ignored.

In the present research, sustainable conditions for "Genovese" basil (*Ocimum Basilicum*) indoor horticulture were considered as well, as it is one of the plant species most cultivated in horticulture due to its high nutritional value and cultivation density [26]. As a first innovative aspect, with respect to previous literature, the germination phase was considered in this study. In addition, specific LED light recipes of hyper red and deep blue were investigated, also in combination with different soils and the addition of a specific controlled-release fertilizer based on nitrogen, phosphorus, and potassium macronutrients (NPK). Moreover, in a circular economy perspective, this controlled-release NPK fertilizer was derived from agri-food, industrial, and post-consumer activities as previously described in the literature [27,28]. In the first part of the experimental procedure, different substrates with a negligible quantity of fertilizers were tested to identify the most promising one capable to clearly highlight the effect of light source only on the plant germination and growth. In the second part, the fertilizer effect was considered in combination with LED lights recipes, employing a design of experiments (DoE) approach leading to a reduction of the number of experiments needed to derive statistically reliable correlation among data [29].

2. Materials and Methods

2.1. Materials

For this study, basil (*Ocimum Basilicum*) seeds belonging to the variety called "Genovese" (Producer: Magnani Sementi) were employed as reference. In the first part of the experimental procedure, related to soils with a negligible quantity of fertilizers, three substrates were considered: Irish peat (Vigorplant Italia S.r.l., Fombio, Italy), Vegetal soil (Dal Zotto S.r.l., Fombio, Italy), and Organic potting soil for aromatic plants (OBI Smart Technologies GmbH, Wermelskirchen, Germany). In the second part of the study, with the aim of considering the fertilizer effect, a soil with considerable quantity of fertilizers was considered: Floradur B pot coarse universal potting soil (Floragard Vertriebs GmbH, Oldenburg, Germany). Detailed specifications of these substrates are reported in Table 1.

Table 1. Substrates properties as indicated by the different manufacturers.

Property	Irish Peat	Vegetal Soil	Organic Soil	Floradur B
Apparent density (kg/m^3) (fresh matter)	200	300	320	120
Electrical conductivity (mS/cm)	0.0058	0.0053	0.0062	0.3800
pH	3.5	6.5	6.8	6.1
Salt content (kg/m^3)	-	-	1.5	1.2
N (kg/m^3)	0.70	-	0.05–0.30	0.21
P_2O_5 (kg/m^3)	-	-	0.08–0.30	0.12
K_2O (kg/m^3)	-	-	0.08–0.40	0.26

With the aim to evaluate the combined effect of a very specific type of controlled release fertilizer and different light sources, in the second part of this study, a NPK lightweight aggregate from agro-residues, wastes, and by-products resulted from previous research [27,28] was employed and compared with the employment of only soil enriched in fertilizers (Floradur B). According to the literature, the NPK controlled-release fertilizer demonstrated a relevant content of nutrients such as nitrogen (8%), potassium (8%), and phosphorous (5%) [27].

As an alternative to daylight, different LED modules specifically designed for horticulture applications were used. All the considered modules (from Intelligent Led Solutions, [30]) are equipped with 12 Oslon®SSL ThinGan LEDs (UX:3) and driven by a constant 370 mA current driver. The modules exploit a specific lens from LEDiL [31] to reduce the radiation angle of the LEDs from the original 80 deg to about 30 deg to obtain a more constant irradiation for all the plants. The considered modules use hyper red (HR) and deep blue (DB) LEDs with 660 nm and 451 nm wavelength at the emission peak, and full width at half maximum (FWHM) values of 25 nm and 20 nm, respectively [32,33]. Three different light recipes have been obtained exploiting modules with different ratios in terms of number of LEDs per type; see Table 2.

Table 2. Hyper red–deep blue light-emitting diodes (LEDs) ratio for the considered light recipes.

LEDs Ratio (Light Recipe Code)	Number of HR LEDs	Number of DB LEDs
3HR:1DB	9	3
1HR:1DB	6	6
1HR:3DB	3	9

All the three considered LED modules have been experimentally characterized by means of a low-cost spectral sensor (AS7341 from Ams, [34]) to obtain a qualitative comparison of the light spectrums provided. The results are shown in Figure 1.

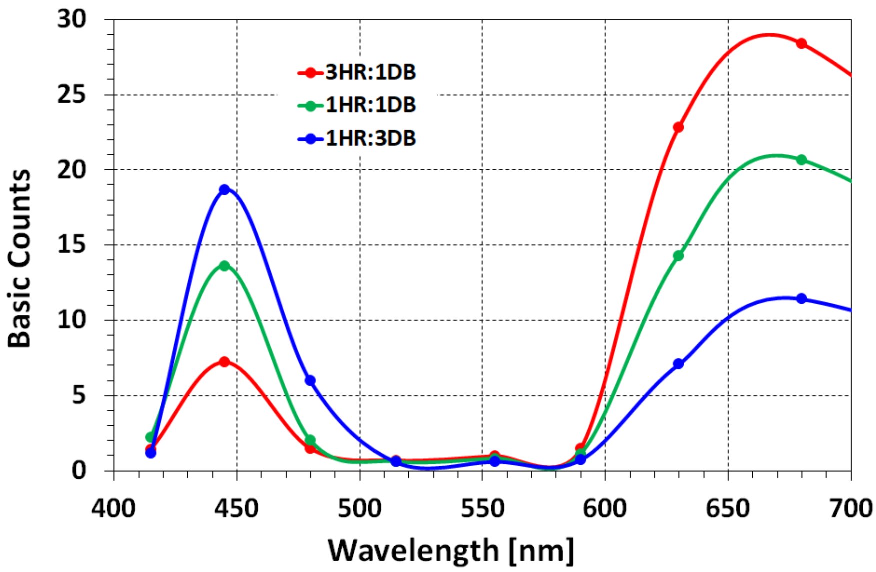


Figure 1. Comparison of the qualitative light spectrums for the considered modules: 3HR:1DB, 1HR:1DB, and 1HR:3DB, obtained using the low-cost AS7341 spectral sensor. Each curve is obtained by the interpolation of the dimensionless output values of the eight optical channels of the sensor with filters centered at wavelengths of 415, 445, 480, 515, 555, 590, 630, and 680 nm, respectively (marker dots). DB: deep blue, HR: hyper red.

More in detail, the used sensor has a sensing element comprised of a matrix of photodiodes used in combination with embedded selective filters to obtain an 11-channel spectrometer. The filters are embedded using nano-optic deposited interference filter technology, and its package provides a built-in aperture to control the light entering the sensor array. Eight of the 11 available channels are reserved to the visible spectrum with filters centered at wavelengths of 415, 445, 480, 515, 555, 590, 630, and 680 nm, respectively. In addition, it has an optical channel dedicated to measure near-infrared light (NIR), an optical channel with a photodiode without filter (called "clear"), and a dedicated channel to detect 50 or 60 Hz ambient light flicker. Light-to-frequency converters embedded into the sensor are used to convert the analog optical channel's output of the photodiodes to dimensionless digital output values of the sensors indicated as basic counts.

Concerning the growth of plants one of the most important parameters is the Photosynthetic Photon Flux (PPF) emitted by the light source used (i.e., the LED modules in our case). PPF refers only to the photons with wavelengths in the range 400–700 nm, because they are the only photons that contribute to the photosynthesis of the plants. Consequently, qualitative light spectrums shown in Figure 1 have been obtained by the interpolation of only the sensor output data provided by the 8 optical channels covering the visible spectrum.

It is important to note that the photosynthetic photon flux (PPF) composition does not reflect the number of LEDs per type ratio of the light recipes. The reason is that the overall photon flux (*PF*), in µmol/s, depends on the radiant flux (*RF*), in W, and is proportional to the light wavelength, λ, (1). Consequently, for a given *RF*, the number of photons generated by an HR LED is higher than the one of a DB LED. Vice versa, since the energy per photon, E_{ph}, is inversely proportional to λ, (2), DB LEDs have a higher *PF* than HR LEDs for a given number of photons.

$$PF = RF \cdot \lambda \cdot 0.00836 \quad (1)$$

$$E_{ph} = h \cdot (c/\lambda) \quad (2)$$

where the coefficient $0.00836 = 1/(h \cdot c \cdot N)$, h is Planck's constant, c is the speed of light, and N is the number of Avogadro.

In addition, both RF and PF depend on the current flowing through the LEDs. For all the considered modules, all the embedded LEDs were connected in series; therefore, they were driven by the same current and this means that HR and DB LEDs provide a different contribution to the total PPF, as shown in Table 3. The data shown in Table 3 were calculated combining the measurements carried out with the low-cost spectral sensor, direct measurements of power supply voltage and current of the LED modules carried out with digital multimeters, and the data reported in the datasheets of the modules [30] and the datasheet of the LEDs embedded into the modules [32,33]. Temperature and relative humidity were recorded using wireless sensors with accuracy equal to ±1 °C (resolution: 0.1 °C) for temperature and ±5% (resolution: 1%) for humidity.

Table 3. Photosynthetic photon flux (PPF) composition.

Light Recipe Code	PPF HR LEDs [µmol/s]	PPF DB LEDs [µmol/s]	Total PPF [µmol/s]	%PPF HR	%PPF DB
3HR:1DB	18.13	6.79	24.92	72.76	27.24
1HR:1DB	12.08	13.57	25.65	47.10	52.90
1HR:3DB	6.04	20.36	26.40	22.88	77.12

2.2. Germination and Growth Trials without Fertilizer Addition

The aim of these trials was to determine the compatibility of basil with three substrates without fertilizer (Irish peat, Vegetable soil, and Organic soil) and the effect of the LED light source employment. Approximately 90 basil seeds per unit were laid in cylindrical nonwoven containers with a volume of 165 cm^3 with 6 repetitions for each soil for a total of 18 units. Trials lasted 20 days, at the end of which the number of plants grown, their mass, and average height were evaluated on randomly sampled plants, 10 for each substrate type. Taking into consideration the most promising soil, 6 repetitions were performed for each light source: daylight, 3:1, and 1:3 HR:DB ratios separately. The photoperiod was set to 15 h/day for 30 days. Humidity in the culture room was maintained between 60 and 80% through irrigation with distilled water daily as required during the experimental period. Artificial light treatments were applied using a growth structure (Figure 2) divided into different sectors. Each sector was separated from external sources of light through fixed wood panels as walls. Plants were placed on the bottom in a fixed position, while LED modules were placed on an adjustable system to ensure a constant height equal to 40 cm and a constant illumination of the top canopy of basil in an area equal to 400 cm^2.

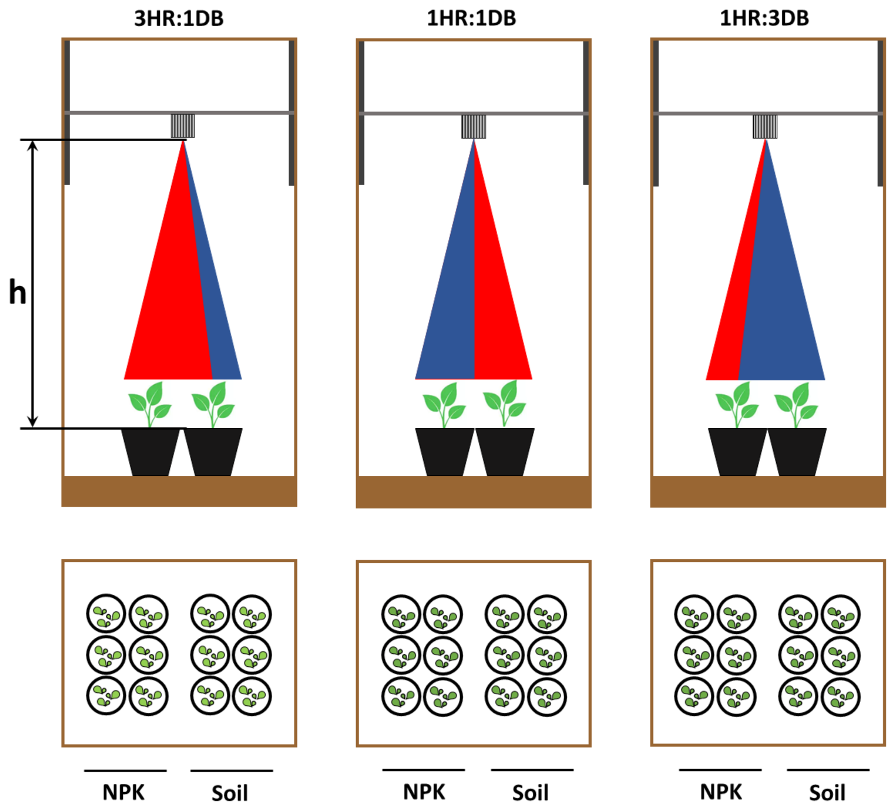


Figure 2. Simplified sketch of the experimental setup: front view (**top**) and top view (**bottom**).

2.3. Design of Experiments (DoE)

A rational approach, codified by the design of experiments (DoE), was employed to obtain the highest amount of information using the minimum number of experiments, saving time and costs [29]. In the second part of the present study, the synergic effect of the amount of the LED light and controlled release fertilizer was investigated on the germination and growth performances. Two factors were considered: NPK as categoric (2 levels: YES/NO) and HR:DB ratio as discrete numeric (3 levels: 1:1, 3:1, and 1:3). The other variables occurring in the process and not specifically considered in this study, such as temperature, humidity, and growth media, were kept constant during all the tests, according to the procedure as explained in Sections 2.1, 2.2 and 2.4. The Design Expert 12.0 (Stat-Ease) code was used both to set up the experimental plan and to analyze the results. Due to the limited number of factors, a full factorial design was selected. A total of 36 experiments were performed including three repetitions for pure error estimation; thereafter, 12 different combinations among NPK and LED lights were observed (Table 4). Central points, considered as the arithmetic average of the factors' levels, were included to investigate the presence of curvature in the data analysis. All the experiments (runs) were carried out randomly to avoid the presence of systematic errors, following the method reported in Section 2.4.

Analysis of variance (ANOVA) was used to point out the cause–effect relationship between controlled-release NPK fertilizer and LED lights on the germination and growing performance. The main assumptions of the ANOVA are that each input factor is independent from each other, normally distributed, and that the variation of the response can be decomposed into different components to evaluate the effect of each factor, their interactions, and experimental error (or unexplained residual) [29]. Through the F-test, variation among all the samples, usually due to process difference or factor changes, is estimated as larger or not than the variation within samples obtained in same experimental conditions. The *p*-value is the statistical parameter used to evaluate the significance of the model and of each factor and represents the probability that the considered model or factor is significant

(p-value < 0.05) or not [35]. The quality of the fit in terms of regression analysis and the predictive power of the model were evaluated by using the R^2 and Pred-R^2, respectively. R^2 is the proportion of the variance in the dependent variables that is predictable from the independent variables, and Pred-R^2 is analogous but associated with predicted values [36].

Table 4. Experimental plan results.

Run	HR:DB	NPK	Height (cm)	NoL	TFM (g)	TDM (g)	LAI (%)	SLA (cm^2/g)
1	3:1	yes	14.63	9.0	4.641	0.353	7.56	566.70
2	3:1	yes	13.33	8.4	3.931	0.293	8.17	601.84
3	3:1	no	12.69	8.0	2.346	0.217	4.56	463.71
4	3:1	no	14.41	8.0	2.772	0.259	4.20	448.71
5	1:1	yes	16.96	11.0	8.124	0.563	6.48	601.78
6	1:1	yes	12.09	8.0	4.512	0.328	7.06	542.05
7	1:1	no	12.17	7.0	2.772	0.285	4.12	422.19
8	1:1	no	12.11	7.6	2.187	0.210	4.23	439.59
9	1:3	yes	14.94	9.3	5.518	0.373	6.60	679.11
10	1:3	yes	15.09	10.7	6.128	0.407	7.42	637.12
11	1:3	no	11.84	8.0	2.452	0.208	4.82	523.50
12	1:3	no	11.33	7.6	2.174	0.192	4.23	479.51

2.4. Growth Experiments with Fertilizer Addition through DoE

Basil seeds were buried in Floradur B soil in plastic pots with a volume of 600 cm^3 and surface area of 78.5 cm^2. Following the experimental plan in Table 4, 36 buried plants were employed, and in some of them, NPK aggregates were included in group of 10. This quantity was determined by considering the 2018 Integrated Production Regulations of the Emilia Romagna Region (Italy) [37] for the production of basil. According to it, under standard soil conditions, the quantity of allowed nutrients such as nitrogen phosphorus and potassium (NPK) are 100–70–80 kg/ha, respectively. In particular, considering the nitrogen amount in the coating, the amount of nitrogen provided is equal to the maximum allowed according to the above-mentioned guidelines and previous NPK characterization, assuming a final release of 50% of the nitrogen contained in the coating [27].

Plants were exposed to the action of the LED modules at a constant distance of 40 cm to ensure homogeneous illumination in an area of 20 × 20 cm. The distance remained constant throughout the experiment, raising the modules as the seedlings grew. The photoperiod was set to 15 h/day for 30 days. The temperature and relative humidity of the samples were monitored during the experiment using one wireless sensor for every set of samples under the same LED module as described in Section 2.2.

2.5. Characterization

At the conclusion of each trial, the following properties were measured for each plant: Total Fresh Mass (TFM), Total Dry Mass (TDM), Height, and Number of Leaves (NoL). For each leaf, fresh mass and area were measured. By adding up all the masses and areas of the same plant, the Total Leaves Mass (TLM) and Total Leaves Area (TLA) were calculated for each plant. Average Leaf Mass (ALM) and Average Leaf Area (ALA) were calculated as well, (1) and (2) formulae:

$$ALM = TLM/NoL \tag{3}$$

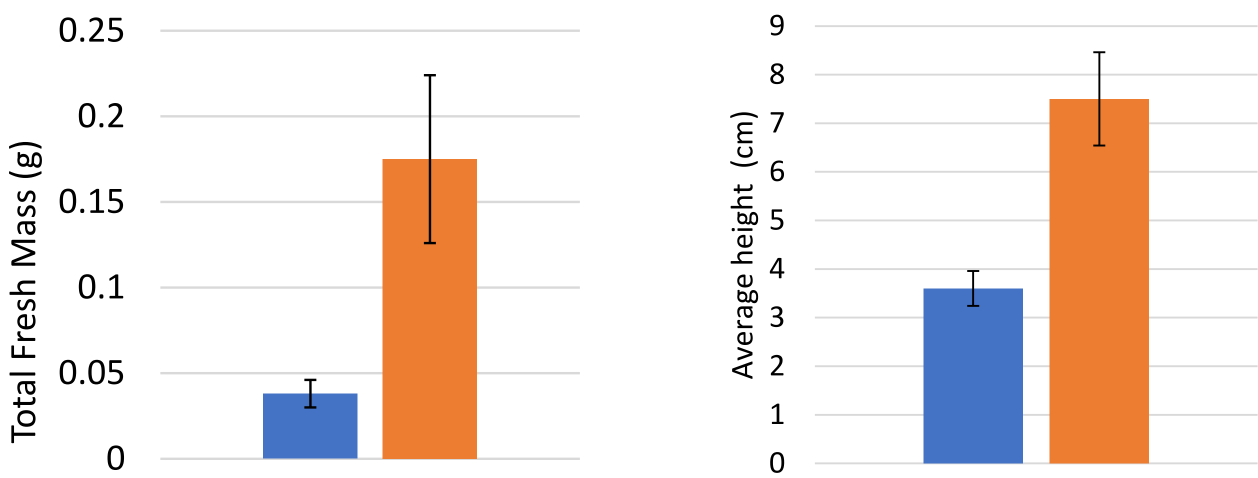
$$ALA = TLA/NoL \tag{4}$$

For masses, a laboratory balance (G&G GmbH, model PLC200B-C) with sensitivity ±0.001 g was employed, and for heights, a digital caliper (Borletti CDJB15-20 series) with a resolution of 0.01 mm and accuracy of ±0.02 mm was used. For dry mass measurement, plants and leaves were dried at 80 °C for 24 h. Leaf area measurement was performed by scanning each leaf at 400 dpi on graph paper and measuring using ImageJ software (version 1.52, NIH, Bethesda, MD, USA). Prior to scanning, leaves were cut at certain points to extend their full area on the paper and to better assess their area. Following these measurements, the LAI (Leaf Area Index), given by the ratio of Average Leaf Area to the area of the pot in which the plants had grown, and the SLA (Specific Leaf Area) index, given by the ratio of Average Leaf Area to Average Leaf Dry Mass, were also calculated [22,38].

3. Results and Discussion

3.1. Germination and Growth Trials without Fertilizer Addition

As can be seen in Table 1, Irish peat is distinguishable for its low pH value with respect to the other soils, but the density and content of fertilizer (negligible) are comparable with the others soils without fertilizer. Organic soil compared with Vegetal soil shows a slight content of fertilizer and thereafter a moderate increase in electrical conductivity. Floradur B, on the other hand, is distinguishable due to the relevant concentrations of the three main plant nutrients: nitrogen, phosphorus, and potassium. Concerning conductivity, for all the soils, the limit generally applied to fertilizer compounds necessary to be sold and employed was respected (<2 mS/cm) [39]. At the final harvest, after 30 days under daylight, plants grown in the different the three potting soils without relevant content of fertilizer showed different performances to basil germination and growth. For the first, it must be noted that the employment of Vegetal soil leads to the germination of only one plant, that is a very restrained value when compared to Irish Peat and Organic soil, which were capable of induce the germination of 61% and 75% of the buried seeds, respectively. This result is probably due to the fact that Vegetal soil is the only substrate with a complete absence of nutrients and salts, which is necessary for the plant germination. Thereafter, due to the high incompatibility of Vegetal soil with basil, this substrate was withdrawn from further investigation and characterization. Figure 3 shows the comparison among two principal growth properties related to Irish peat and Organic soil employment: the average height and TFM of the plants. These results highlight that although Irish peat showed excellent compatibility with basil, Organic soil is the most promising soil in terms of TFM and average height of the plants in reference to a quite similar number of sprouted plants. This result is compatible with the fact that Organic soil contains a small amount of nutrients capable of acting as fertilizers and a pH nearer to values more compatible with basil cultivation [40].



(**a**) (**b**)

Figure 3. Comparison of Irish peat (in blue) and Organic soil (in orange) considering: (**a**) Total Fresh Mass and (**b**) Average height. Error bars indicate the standard deviation on the average values.

Taking into account the employment of only Organic soil, it has been demonstrated that the light spectrum strongly affects plant growth and development; in fact, basil plants grown under LED lights modules generally show better performances than the plants exposed to daylight, as shown in Table 5 and Figure 4. This is particularly true for parameters such as Height, TFM, TLM, and ALM. On the other hand, the results obtained for the parameters NoL and TDM do not highlight any specific trend. Regarding the comparison between plants grown under two different LED lights combinations, no statistically significant differences emerged. In fact, although plants grown under the 3HR:1DB module showed generally higher mean values of the growth parameters than 1HR:3DB, the standard deviation ranges suggest a similar behavior of the basil plants growth among the two combinations employed. These results are also suggested by a visual comparison of the plants obtained through the three enlightenment methods (Figure 4). For the SLA index, the highest value is guaranteed by 1HR:3DB employment, and this is particularly important because this index depends on the growth environment of the plants and describes their degree of adaptation to it from a morphological point of view. For the LAI parameter, no difference among the two LED module employments can be noted, even if a substantial increment of this index is observed by moving from daylight to LED enlightenment. This parameter is used to determine the size of the interface between the plant and atmosphere, thus the exchange of mass and energy between them to model the photosynthesis mechanism. In addition, it must be noted that a control group of plants grown without any type of light has been tested, obtaining a not significant number of plants without leaves. Thereafter, for this control group, only the plants' height was measured and equal to 2.03 ± 0.62. These results suggest that the LED light combinations investigated in this study play a valuable role as a different source of enlightenment from daylight to improve the basil growth performance in condition with a negligible quantity of nutrients beneficial for cultivation.

Table 5. Growth performance for basil plants under different lights.

	Daylight	3HR:1DB	1HR:3DB
Height (cm)	6.60 ± 1.09	6.11 ± 1.03	9.28 ± 0.96
LoN	7.0 ± 1.4	6.0 ± 0.0	5.6 ± 0.5
TFM (g)	2.895 ± 0.630	5.788 ± 0.392	5.145 ± 0.488
TDM (g)	0.388 ± 0.201	0.589 ± 0.038	0.465 ± 0.072
TLM (g)	2.330 ± 0.496	4.914 ± 0.349	4.308 ± 0.503
ALM (g)	0.357 ± 0.037	0.819 ± 0.058	0.769 ± 0.054
TLA (cm^2)	72.73 ± 10.99	110.48 ± 10.33	106.12 ± 11.11
ALA (cm^2)	9.70 ± 0.71	18.41 ± 1.72	18.98 ± 1.83
SLA (cm^2/g)	209 ± 56	224 ± 18	282 ± 37
LAI (%)	2.7 ± 1.1	5.1 ± 0.5	4.9 ± 0.5

DAYLIGHT **1HR:3DB** **3HR:1DB**

Figure 4. Basil plants at the end of the growth test performed under different lights and with the Organic soil as substrate.

3.2. Growth Experiments with Fertilizer Addition through DoE

With the aims of finding an optimal LED lights combination, balancing the number of hyper red and deep blue LED lights, that is capable of enhancing all the growth parameters, and investigating the possible effects in combination with fertilizer, a new experimental set-up was managed through an ad hoc DoE experimental plan. Table 4 shows the complete experimental plan and the obtained results, in which the average values of each response among three repetitions of the same experiment are indicated.

As previously stated, with the aim to evaluate only fertilizer and LED lights effects, all the other parameters were kept as constant; thereafter, temperature and humidity were controlled during all the experiments to avoid environmental conditioning. Temperature and humidity were kept almost constant near 30 °C and 60% respectively, with restrained variation around 5% due to the employment of an indoor environment not perfectly conditioned. From a general observation of the results, germination occurred for almost all the basil seeds; in fact, only two of them did not germinate, and thereafter, the number is not statistically relevant. In addition, it must be noted that all the cultivation conditions applied according to this experiment plan were proved to be appropriate for basil, as it was confirmed by the absence of morphological and developmental abnormalities during plant growth. Observing only the data in Table 4, a general comparison can be stated with the results obtained without fertilizer, as shown in Table 5. From this comparison, it is clear that the fertilizer addition, by soil or by controlled-release fertilizer addition, is capable of improving the basil growth performances. In particular, it can be observed that plant height is always greater with fertilizer as well as the NoL and SLA parameters. Figures 5–7 show examples of the plants obtained at the end of the experimentation, respectively those grown under 3HR:1DB, 1HR:1DB, and 1HR:3DB, thereafter moving from a higher to lower proportion of hyper red LED light in relation to deep blue. As can be seen observing Figures 5–7 and the data in Table 4, the introduction of NPK controlled-release fertilizer has a positive effect on the plants' growth by increasing a considerable number of plants parameters (such as Height, TFM, TDM, LAI, and SLA), and in general, this effect is not influenced by the type of LED light. Nevertheless, from Figure 7, it appears clear that a more distinguishable difference among plants with and without NPK is appreciable, suggesting a possible synergic effect between NPK addition and 1HR:3DB LED lights combination that is capable of possibly promoting plant height and TFM. For these reasons, it is essential to study the ANOVA analysis to mathematically identify the possible correlation among NPK and LED lights combination as a function of the different measured parameters.

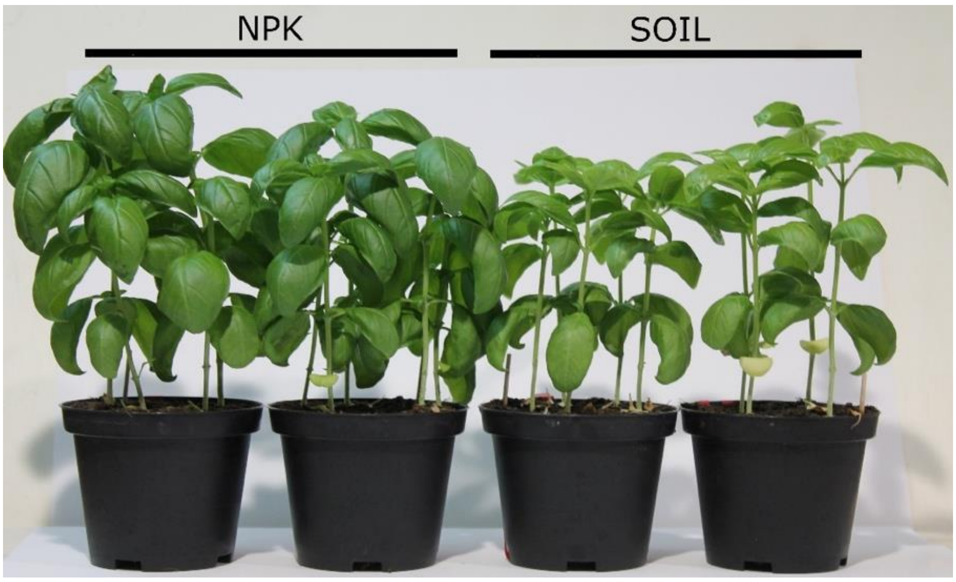


Figure 5. Basil plants obtained under 3HR:1DB LED lights.

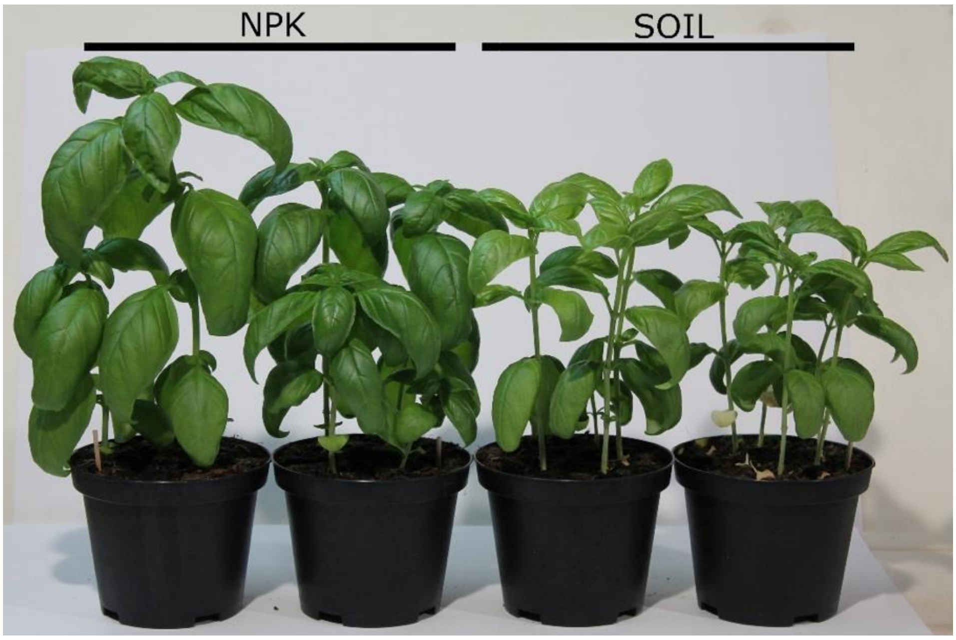


Figure 6. Basil plants obtained under 1HR:1DB LED lights.

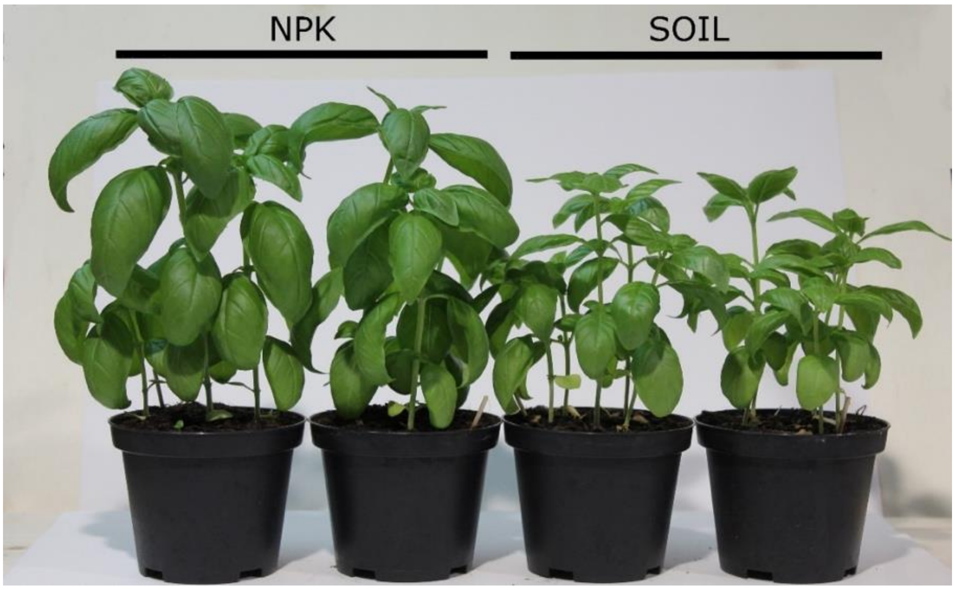


Figure 7. Basil plants obtained under 1HR:3DB LED lights.

The normal distribution of the residuals, as well as their homogeneity, has been analyzed (data not reported) for each response, confirming that the mathematical models derived can be used to explore the region of interest. The resulting models allowed describing the relationships between growth condition and the measured responses. The ANOVA results have been presented in Tables 6 and 7, where the quantification of the significance of factors and their interactions were reported as well as the fitting quality parameters as well as the effect sizes for each significant model. An inverse square root transformation was required to normalize the data and codify the hierarchy of the factors for some responses. The model correlating the factors (in single or interaction) to the panel data evaluation is significant, as confirmed by the p-value < 0.0001 for all the responses, which means that the probability of the data variation due to unknown factors is statistically irrelevant. Moreover, it is relevant that the curvature is not significant, and therefore, the central points can be treated as additional data in the regression model, augmenting the design plan. R^2 and Pred-R^2 (Table 6) confirm the sufficient fit of the data and a quite fair predictive power for four responses, having $R^2 > 0.45$, with a particular high fitting for SLA parameters with $R^2 = 0.75$. Considering all the models' equations in Table 6 (where the coefficients of the variables in the model are reported), it is clear that NPK controlled-release fertilizer plays the main role in the performance of the responses, and this is consistent with the previous hypothesis, but a synergic effect has been shown and calculated for the SLA responses, which also corresponds to the model with higher fitting quality (higher R^2).

Table 6. ANOVA results.

Response	Transformation	R^2	Pred-R^2	Models' Mathematical Expression NPK = NO	NPK = YES
Height	NONE	0.13	0.10	-	
NoL	NONE	0.47	0.41	= 8.000	= 9.666
TFM	Inverse Square Root	0.58	0.53	= 2.032	= 1.565
TDM	NONE	0.28	0.19	-	
LAI	Inverse Square Root	0.55	0.49	= 0.543	= 0.413
SLA	Inverse Square Root	0.75	0.68	$= 0.045 + 3.7 \times 10^{-5} \times$ HR:DB	$= 0.039 + 3.7 \times 10^{-5} \times$ HR:DB

Table 7. Effect sizes for significant responses.

Response	Intercept	NPK	HR:DB
NoL	8.83	0.8333	-
TFM	1.80	−0.2340	-
LAI	0.47	−0.0615	-
SLA	0.04	−0.0032	0.0009

Figure 8 shows interaction graphs representing graphically each calculated model. A quite similar behavior can be detected for NoL, TFM, and LAI (Figure 8a–c), where an increasing of these responses is observed by introducing the NPK fertilizer independently of the LED lights combination. In contrast, the SLA parameter (Figure 8d) is clearly influenced by a synergic effect of NPK and LED lights: a restrained quantity of hyper red (1HR:3DB) is favorable to the SLA increase, independently of the presence of NPK, even if the highest value of SLA is obtained employing also controlled release NPK. The overall result is that a high quantity of hyper red LED light should be avoided to promote the basil growth. This result is in agreement with previous literature related to other plants' growth in an indoor and black environment such as lettuce, tomato, and flowers, where the employment of a high quantity of red LED light leads to reduced dry weight, plant height, and leaf area [22,23,41,42].

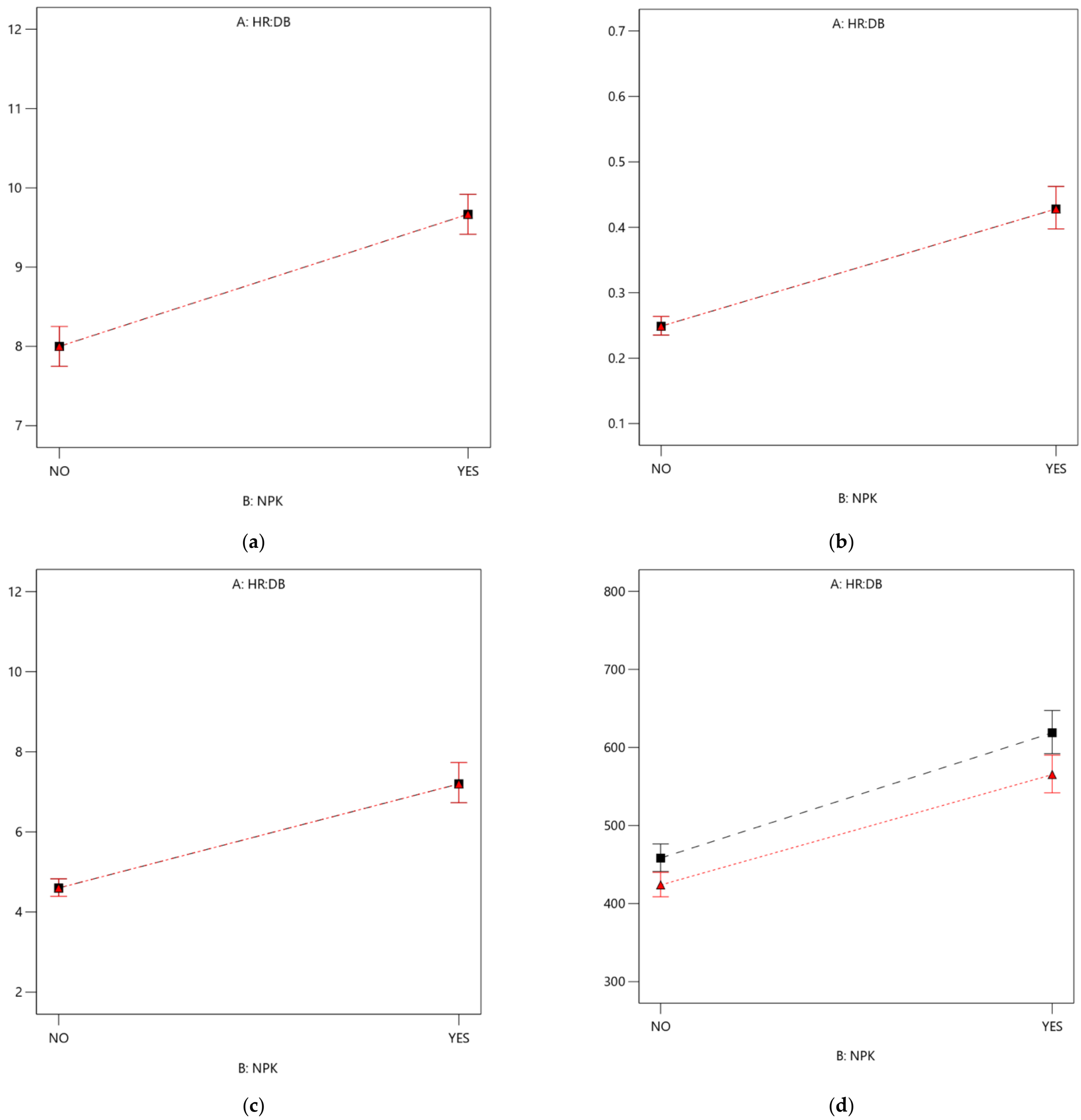


Figure 8. Interaction plots of different responses: in black color, the interaction lines related to 1HR:3DB; in red color, the interaction lines related to 3HR:1DB: (**a**) NoL; (**b**) TFM; (**c**) LAI; (**d**) SLA. Error bars indicate the standard deviation on the average values. LAI: Leaf Area Index, NoL: Number of Leaves, SLA: Specific Leaf Area, TFM: Total Fresh Mass.

4. Conclusions

In this study, it has been demonstrated that the implementation of various combination of LED lights is generally favorable to basil (*Ocimum Basilicum)* germination and growth with respect to daylight (e.g., Height: +30%, Total fresh mass: +50%), although no differences among hyper red and deep blue LED lights combination can be noticed when a soil without any fertilizer is employed as growing media. Applying a design of experiments approach, mathematically reliable information has been derived concerning a possible synergic effect with a soil enriched in macronutrients and with an NPK controlled-release fertilizer derived from agro-residues in a circular economy perspective. It has been demonstrated that the use of NPK controlled-release fertilizer provides better performance, for the cumulative values for individual pots, than the use of fertilized soil alone. In addition, for the SLA parameter, the highest value is obtained by employing controlled-release NPK fertilizer coupled with a specific LED light combination (one hyper red: three deep blue). It should be noted that the present study analyzed the influence of different factors on basil growth considering only one moment of collection without considering the variation of the light recipe in the different phases of life of the same. Thereafter, for a future perspective, the variation of the light recipe during the different phases of life of the basil plant should be investigated. Moreover, by taking some leaves or part of them and storing them in liquid nitrogen, it would be possible to perform biochemical measurements, such as determination of the content of phenolic compounds, flavonoids, antioxidant capacity, nitrate content, chlorophyll content, and carotenoids. Finally, it must be noted that basil is an edible aromatic herb that is extensively employed in the Mediterranean diet; therefore, panel test judging the possible different tastes should be proposed as a future perspective.

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