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Effect of Different LED Light Wavelengths on Production and Quality of *Pleurotus ostreatus* Grown on Different Commercial Substrates

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Abstract: Artificial lighting, primarily employed in crop production, can also be applied to the cultivation of edible mushrooms to enhance productivity and quality. While UV radiation has predominantly been investigated in post-harvest treatments for edible mushrooms, the utilization of different light wavelengths during the cultivation phase remains largely unexplored for many mushroom species. This study aimed to assess the impact of three different light wavelengths 450 nm (B), 610 nm (R), and a combination of these two wavelengths (R + B) on the productive characteristics and quality of *Pleurotus ostreatus*, cultivated using three straw-based commercial substrates. It was observed that, except for yield, artificial light influenced mushroom growth. Specifically, the application of R light appeared to promote mycelium growth, whereas B light contributed to increase the diameter of fruiting bodies. Additionally, the concentration of vitamin D_2 was higher under both B and R+B light treatments. Interestingly, the light treatments did not affect yield but impacted diameter and various chemical attributes such as EC, total soluble solids, and titratable acidity. In conclusion, exposure to different lighting affected *Pleurotus ostreatus* physiology and nutritional content.

Keywords: artificial lighting; edible mushrooms; yield; vitamin D

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1. Introduction

Edible mushroom production has been increasing in recent decades, reaching approximately 40 million tons globally [1]. Meanwhile, the consumption of edible mushrooms grew from 1 kg to 4 kg per capita in the last 15 years [2]. China and Asian countries are the largest producers worldwide, followed by the USA and EU [1]. The most important species cultivated industrially belong to the Basidiomycetes and are saprophytes: *Agaricus bisporus* (button mushroom) occupies 38% of global production, *Pleurotus* spp. (oyster mushrooms) accounts for 25%, and *Lentinula edodes* (shiitake mushrooms) accounts for 10% [3]. Many important species belong to the *Pleurotus* genus, such as *P. ostreatus*, *P. pulmonarius*, and *P. eryngii*. They are characterized by significant nutraceutical traits; they are high in protein (19–35%), contain 9 essential amino acids [4], and are also a good source of vitamins B, C, and D_2 [5]. *Pleurotus* spp. are primary decomposers and are known for their high degradation capacity and ability to produce a wide range of extracellular enzymes for lignin, cellulose, and hemicellulose degradation [6,7]. For this reason, these species can colonize and degrade a variety of substrates derived by agro-industrial waste, and they require a short growing period [8]. *P. ostreatus* is one of the most important species produced worldwide [9], and in Europe it is cultivated mainly on wheat or rye straw substrate [10]. The cultivation process can be either in bags or bottles filled with substrate [7,11]. *P. ostreatus* is one of the least difficult species to cultivate [11]; the substrate can be prepared

quickly and it does not need complex technology for production [12]. For this reason, its cultivation is spreading in many developing countries [13]. Environmental conditions affect the production and quality of fruiting bodies of *Pleurotus* species, however, it can grow in a wide range of temperatures, which also makes it suitable for production in tropical climate conditions [11]. Light radiation is an abiotic factor that can affect the productive and qualitative traits of *Pleurotus* spp. Unlike *A. bisporus*, mushrooms belonging to *Pleurotus* genus require light during the fruit body formation, and a phototropism behaviour has been observed [14]. The amount of light needed by *Pleurotus* spp. for promoting fruit body formation is between 4.6-17.7 µmol m^{-2} s^{-1} with a photoperiod of 8-12 h day^{-1} [15]. A dark environment could cause deformation in fruiting bodies, stipe elongation, and a sub-optimal coloration of the cap [15]. Light can also improve the qualitative characteristics of edible mushrooms during the growing stages, regulating physiological and nutritional metabolism, and in the post-harvest period, affecting shelf life [16]. Artificial light can enhance the effects during both pre- and post-harvest stages. Previously published studies focused primarily on the effects of UV radiation on post-harvest mushrooms, highlighting the ability to increase the vitamin D_2 content in *A. bisporus*, *L. edodes*, and *P. ostreatus* [17–20]. Few published studies document the effects of artificial lighting, using specific wavelengths, during the cultivation of edible mushrooms [16]. Current knowledge focuses on the effects of different wavelengths and intensities of radiation at a molecular level to understand how light impacts metabolic pathways and gene expression during the mycelium's growth. During cultivation, only a few mushroom species, primarily *P. eryingii*, have been observed under different light treatments [21]. Previous studies reported results from exposure in Petri dishes or incubators, demonstrating that blue light (450-500 nm) can improve cap diameter and promotes a darker fruit body color in *L. edodes*, *P. ostreatus* [22], *P. eryngii*, and *Coprinopsis cinereus* [16], whereas red light (610–760 nm) improves mycelium growth in *P. eryngii* [23]. Only Miyazaki et al. and Yu et al. reported on the effect of different artificial wavelengths under growing conditions in a cultivation room. They demonstrated how both wavelength and radiation intensity affected *P. eryngii* morphological characteristics of fruiting bodies [21,24]. Yu et al. also reported that artificial light improved the nutritional value of mushrooms, enhancing the biosynthesis of some essential amino acids when cultivated under red wavelength lighting [21]. Many experiments were focused on the effect of a single wavelength, mostly in a controlled environment such as a laboratory or growing chamber; no studies looked at the effect of a combination of wavelengths on mushroom development. An industrial LED lighting system already used in horticulture for greenhouse and indoor production could be easily applied to a cultivated mushrooms production system due to the high performance of this type of lighting, which allows for small size, low heat emission, and the simple modulation of wavelength and high energy conversion efficiency [25]. There is a lack of information about the industrial application of artificial light systems with LED during the cultivation stage of edible mushrooms. For this reason, the effect of different light wavelengths was observed during a production cycle of *P. ostreatus* in an industrial mushroom facility.

2. Materials and Methods

2.1. Experiment Set-Up

P. ostreatus cultivation took place in a mushroom greenhouse for *Pleurotus* spp. cultivation at the Experimental Farm "L. Toniolo" at the University of Padova (Northern Italy (45°20′ N, 11°57′ E, 6 m a.s.l.) between September and December 2021. The experiment consisted of three commercial substrates widely used by European Countries, composed of wheat straw and inoculated with P80 strain of *P. ostreatus* (Italspawn, Treviso, Italy). During the incubation period, the air temperature was set at 22 °C, whereas during the cultivation stage, the temperature was maintained at approximately 16 °C, and the concentration of CO_2 inside the mushroom's facility was maintained under 550 ppm. During cultivation, bags were placed on the floor in separated areas supplied with 4 different types of light: red light (R—610 nm), blue light (B—450 nm), a combination of these two wavelengths

(R+B—70% R and 30% B), and natural lighting (C) of the greenhouse. The average light intensity to the bags, measured in different points with the spectroradiometer (Delta Ohm HD 30.1), ranged between 30 and 50 µmol m^{-2} s^{-1}. The duration of light exposure followed the natural photoperiod of the fall season: 8 h on, 16 h off. For each treatment, 9 bags and three replications were considered.

2.2. Substrate Chemical Characterization

At the beginning of the cultivation cycle, a sample was taken to characterize each substrate. A dried representative mixed sample was used to determine the concentration of: (1) total organic nitrogen (TKN) using the Kjeldahl method; (2) phosphorus (P), potassium (K), chromium (Cr), cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) by inductively coupled plasma optical emission spectrometry (ICP-OES) on a Spectro Ciroscd ICP (Spectro Analytical Instruments, Kleve, Germany) [19]; and (3) anions (Cl^-, Br^-, $NO_3{}^-$, $NO_2{}^-$, $PO_4{}^{3-}$, $SO_4{}^{2-}$) and cations ($NH_4{}^+$, Na^+, Mg^{2+}, K^+, Ca^{2+}) using ion chromatography (IC), performed using an ICS-900 system (Dionex Corp., Milan, Italy) equipped with a dual piston pump, model AS-DV autosampler, isocratic column at room temperature, DS5 conductivity detector, and AMMS 300 suppressor (4 mm) for anions and CMMS 300 suppressor (4 mm) for cations. Chromeleon Chromatography Management software (6.5 version) was used for system control and data processing. A Dionex IonPac AS23 analytical column (4 × 250 mm) and guard column (4 × 50 mm) were used for anion separation, whereas a Dionex IonPac CS12A analytical column (4 × 250 mm) and guard column (4 × 50 mm) were used for cation separation. The eluent consisted of 4.5 mmol L^{-1} sodium carbonate and 0.8 mmol L^{-1} sodium bicarbonate at a flow rate of 1 mL min^{-1} for anions and of 20 mmol L^{-1} methanesulfonic acid for cations at the same flow rate. Dionex solutions containing seven anions at different concentrations and five cations were taken as standards, and the calibration curves were generated with concentrations ranging from 0.4 to 20 mg L^{-1} and from 0.5 to 50 mg L^{-1} of standards, as reported by Nicoletto et al. [26].

The substrate chemical analyses of different companies are shown in Table 1.

Table 1. Chemical and mineral characteristics of the three substrates used.

Substrate	Strain	C:N Ratio	Total Nitrogen	P	K	Cd	Cr	Cu	Pb	Zn
			%			mg kg^{-1} dw				
S1	P80	47.5	0.89	989	11,129	<0.1	7.09	5.21	0.97	20.89
S2		60.6	0.70	721	8798	<0.1	11.47	6.37	1.42	19.08
S3		49.3	0.87	813	11,696	<0.1	11.59	5.28	2.68	24.38

2.3. Productive and Qualitative Traits

Different productive traits were monitored during the production cycle; at the beginning of the flush, the emergence of primordia was measured, and the results were normalized to a percentage of fruiting based on the total number of holes per bag (1):

$$\frac{\text{Cumulative production of primordia} \times 100}{\text{Total holes of bag}} \quad (1)$$

During harvest time, from each bag, all the fruiting bodies were collected at commercial maturity stage and weighed. Biological efficiency was calculated with the following formula:

$$\frac{\text{Fresh mushrooms harvest weight} \left(\text{kg} \times \text{bag}^{-1}\right) \times 100}{\text{Dry substrate weight} \left(\text{kg} \times \text{bag}^{-1}\right)} \quad (2)$$

During harvest time, 3 representative clusters of fruiting bodies for each bag were collected to determine the number of fruiting bodies and describe the morphological traits. For three fruiting bodies, the diameter, thickness, and principal colorimetric parameters (L,

a* and b*) were recorded using a tristimulus colorimeter (Chroma Meter CR-410, Konica-Minolta, Milan, Italy). The L value represents darkness and lightness of color in a range between 0 and 100; the a* value, from −60 and +60, is a coordinate to represent greenness and redness, respectively, and the b* value, between −60 and +60, describes the color for blueness and yellowness. Then, L, a*, and b* coordination was converted in RGB to have a visible representation of fruiting bodies' color.

Representative samples for each treatment were collected and frozen at −20 °C, and part of each sample was freeze dried for the measurement of vitamin D_2 content. Portions of the frozen samples were thawed at room temperature, and then tissue moisture was collected by squeezing the samples and used to measure pH, electric conductivity (EC), total soluble solids (°Brix), and titratable acidity according to ISO 750:1998 (E). Vitamin D_2 was measured with the accredited LC–MS/MS method MP 1570 rev 2/2017 [27].

2.4. Statistical Analysis

A randomized block design was carried out, and three replicate bags for each light treatment were utilized. Quantitative and qualitative data were statistically processed by two-way analysis of variance (ANOVA) test, and the means were separated through the Tukey HSD test at $\alpha = 0.05$. Statgraphics Centurion software version 19 (Statgraphics Technologies, Inc., The Plains, VA, USA) was used for statistical processing. PCA was run with Rstudio 4.3.3.

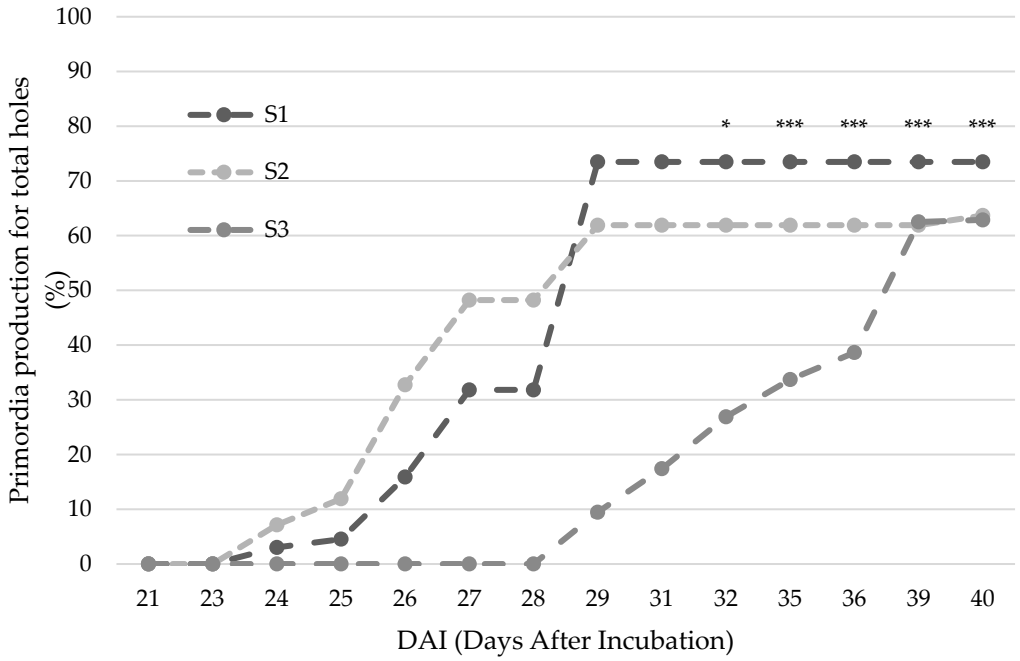
3. Results

3.1. Productive Traits

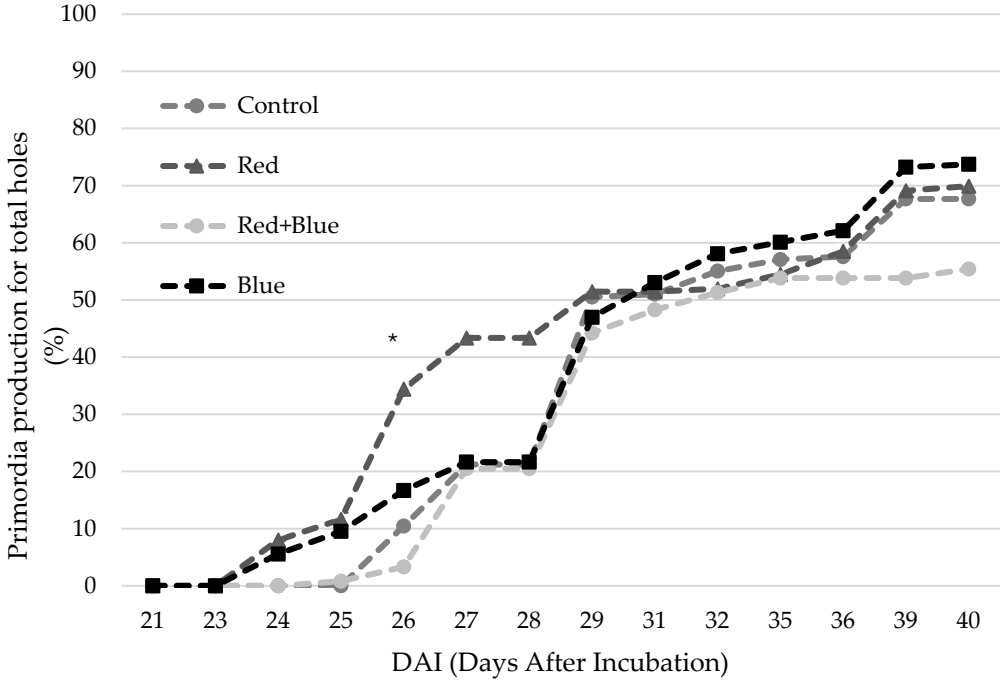
The cultivation substrate affected the timing of primordia formation (Figure 1a). Substrates S1 and S2 developed primordia 23 days after incubation (DAI) compared to S3, which did not develop primordia until 28 DAI. Subsequently, S1 was characterized by a higher production of primordia, reaching 73% by 29 DAI. The percentage of primordia in S2 remained stable between 29 and 40 DAI, with values slightly above 60%, whereas in S3, the primordia growth was slower until reaching S2 values at 40 DAI.

Light wavelength also affected primordia development. Primordia formation started at 24 DAI for all treatments, and the results for both lighting and substrate were significantly different (Figure 1b). Only after 26 DAI, the production of primordia showed a statistical difference with the higher percentage under R light and 32% of holes occupied by primordia. The greater percentage of primordia under R treatment occurred between 26 and 28 DAI. Subsequently, the percentage of primordia was comparable in all treatments until 35 DAI. Between 36 and 40 DAI, only the B, R, and C light treatments reached values close to 70%, whereas the R+B treatment settled at 55%.

Neither light wavelength or substrate significantly affected biological efficiency (Table 2; S2 and R + B had the higher biological efficiency with 61% and 59% respectively, though they were not statistically different. However, both treatments significantly influenced the number of fruiting bodies clusters per kg of substrate. Substrate S1 produced the higher number of fruit bodies with 0.76 clusters per kg of substrate, and the yield of S2 and S3 was lower than 0.50 clusters per kg of substrate. R and R+B lighting treatments increased the number of fruiting bodies clusters. B light alone, however, expressed the lowest result with 0.28 clusters per kg of substrate. Though both treatments impacted the number of fruit body clusters per bag, neither had a significant effect on the number of fruiting bodies per cluster, though a positive trend was observed when exposed to R light with 23.2 fruiting bodies per cluster compared to 21.9 of control light.



(**a**)



(**b**)

Figure 1. Effect of substrate (**a**) and lighting treatments (**b**) on primordia cumulative production percentage expressed on the total holes of the bag. Within each parameter, values with "*" differ at p-value ≤ 0.05, and "***" differ at p-value ≤ 0.001, according to Tukey's HSD test.

Table 2. Effect of substrate and lighting treatments on yield (kg of fresh mushrooms per kg of substrates), biological efficiency (%), nr of cluster for kg of substrate and number of fruiting bodies for cluster. Means are followed by standard error and different letters indicate significant differences among treatments at p-value ≤ 0.05 according to Tukey's HSD test.

	Yield kg kg^{-1} Substrate	Biological Efficiency (%)	Number Clusters kg^{-1} Substrate	Number Fruiting Bodies/Cluster
		Substrate		
S1	0.17 ± 0.01	50.1 ± 2.62	0.76 ± 0.10 a	18.9 ± 0.96
S2	0.21 ± 0.01	61.8 ± 3.03	0.47 ± 0.06 b	19.8 ± 0.90
S3	0.18 ± 0.02	53.1 ± 5.27	0.40 ± 0.06 b	21.6 ± 1.49
p-value	ns	ns	*0.0022*	ns
		Lighting treatment		
Control	0.19 ± 0.01	56.3 ± 3.44	0.68 ± 0.07 a	21.9 ± 1.33
Red	0.19 ± 0.01	54.5 ± 2.68	0.66 ± 0.08 a	23.2 ± 1.58
Red + Blue	0.20 ± 0.01	59.13 ± 3.18	0.56 ± 0.07 ab	18.3 ± 0.82
Blue	0.17 ± 0.03	50.14 ± 7.48	0.28 ± 0.12 b	20.0 ± 1.14
p-value	ns	ns	*0.0043*	ns

The main morphological characteristics detected for fruiting bodies were the diameter and thickness, as shown in Figure 2a,b. The substrate had a statistically significant effect on diameter with a higher value (10.73 cm) for substrate S3 (Figure 2a), whereas for lighting treatments, B wavelength showed higher value compared to the control; the average diameter under B light was 10.74 cm, whereas under R+B and R light the diameter was 9.8 and 9.5 cm, respectively. Thickness was not affected by substrate and lighting treatments (Figure 2b); the maximum value was recorded for substrate S3 (3.67 mm), and under B light it was 3.73 mm.

The visible difference in fruiting bodies color is showed in Figure 3. The main colorimetric parameters (L, a* and b*) of the fruit bodies are reported in Table 3, and only L* and a* were statistically significant. L parameter reached a higher value in fruiting bodies cultivated on S1 and S2 substrate with values of 61 and 62.5, respectively. Moreover, different lighting exposure had a significant effect on L value: R and C showed higher values (63.6 and 63.8, respectively) if compared to B and R + B light where lower values, 56 and 55.7, respectively, were recorded. For a* parameter, an opposite effect was observed with a higher value for B light (3.91) and a lower one for C and R light. The color range between yellow and B (b*) was not influenced by either treatment.

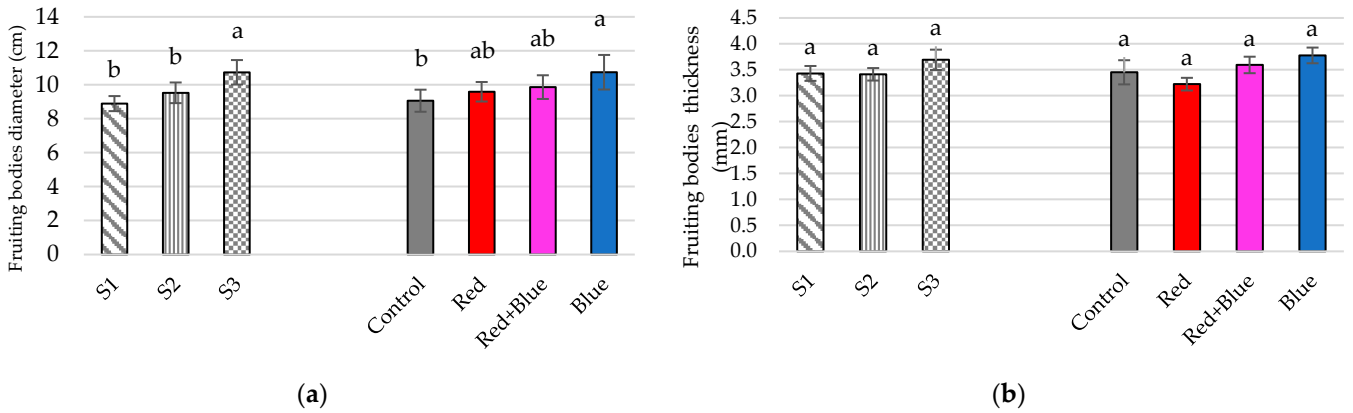


Figure 2. Effect of substrate and lighting wavelength on diameter (**a**) and thickness (**b**) of fruiting bodies. Within each parameter, values without common letters differ at p-value ≤ 0.05 according to Tukey's HSD test. Bars indicate standard error.

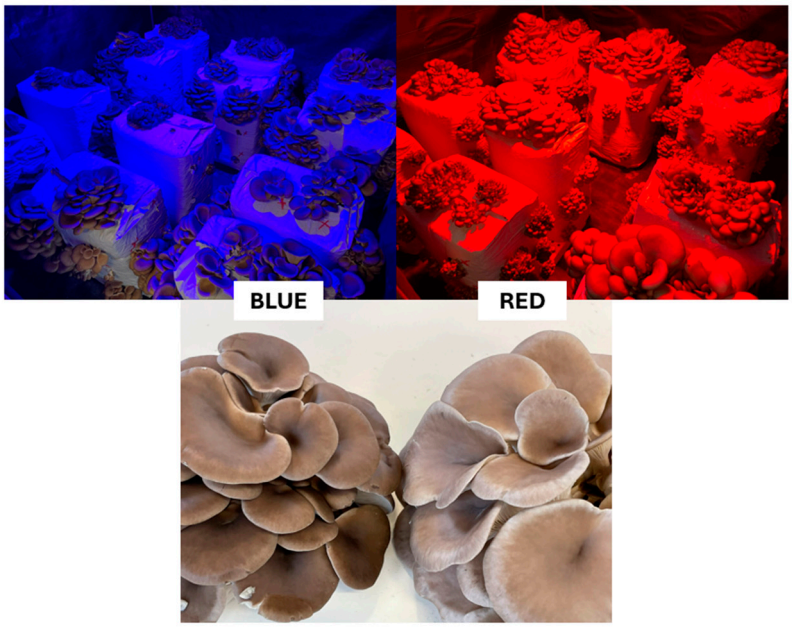
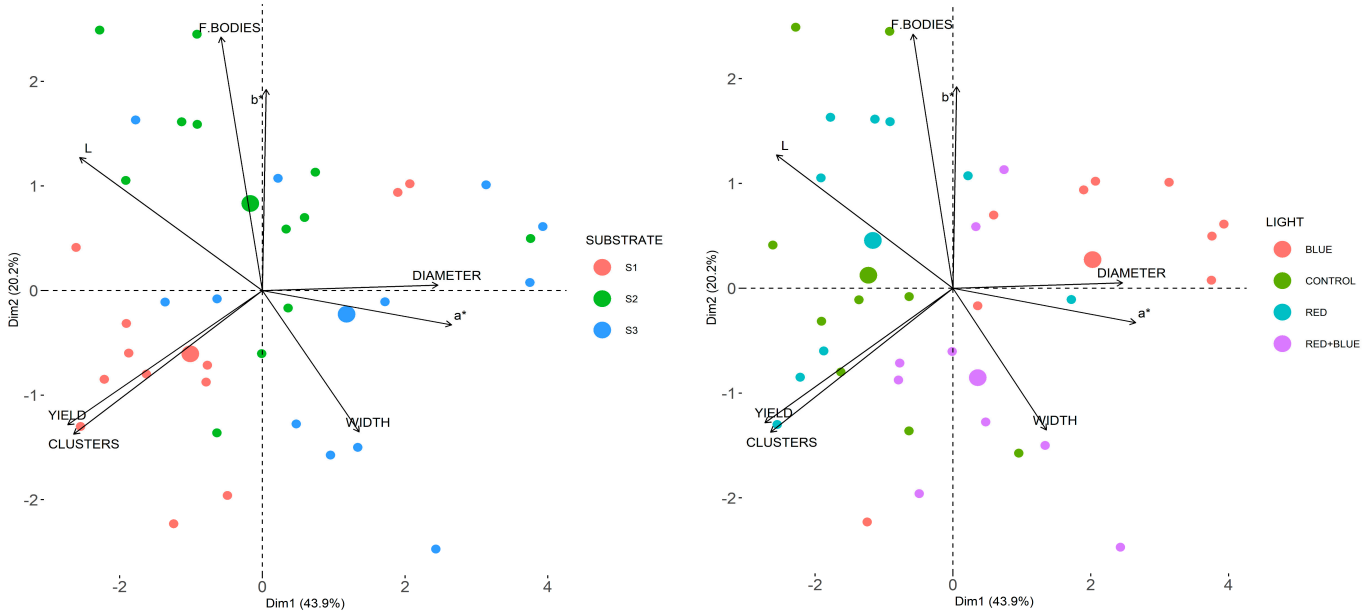


Figure 3. Illustrative images of blue (B) and red (R) cultivation environment and the main difference in fruiting bodies color.

Table 3. Effect of substrate and light treatments on colorimetric parameters (L, a* and b*) of fruiting bodies and the corresponding color. Means are followed by standard error, within the same parameter values without letters in common differ at p-value ≤ 0.05 according to Tukey's HSD test.

	L	a*	b*	Fruiting Bodies Color
		Substrate		
S1	61.0 ± 0.66 a	2.71 ± 0.64 a	9.2 ± 0.41 b	
S2	62.5 ± 0.92 a	2.56 ± 0.70 a	10.24 ± 0.25 a	
S3	56.0 ± 0.74 b	3.29 ± 0.66 b	8.76 ± 0.53 b	
p-value	*0.000*	*0.000*	*0.0001*	
		Lighting treatment		
Control	63.6 ± 0.94 a	2.06 ± 0.15 c	9.55 ± 0.41	
Red	63.8 ± 0.91 a	2.27 ± 0.16 c	9.40 ± 0.25	
Red + Blue	56.0 ± 0.89 b	3.30± 0.16 b	8.94 ± 0.26	
Blue	55.7 ± 1.08 b	3.91 ± 0.17 a	10.03 ± 0.30	
p-value	*0.000*	*0.000*	*ns*	

The PCA results depicted in Figure 4a,b elucidated a variance of 43.9% and 20.2%, respectively, contributing to a cumulative explained variance of 64.1%. The analysis revealed distinct distributions of data points across the three commercial substrates (Figure 4a). Substrate S1 exhibited a cluster of data points in the quadrant primarily characterized by positive correlations between yield and associated loadings. Conversely, substrate S2 occupied the first quadrant, with its distribution largely explained by the loadings associated with the number of fruiting bodies. In Figure 4b, a notable proximity between clusters R and C was observed, in contrast to the distinct positioning of cluster B, which resided in the positive quadrant of both the first and second components. This positioning of cluster B was primarily attributed to the loadings associated with diameter.



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

Figure 4. PCA of substrate (**a**) and light effect (**b**) on production and morphological characteristics (yield, n° cluster, n° fruiting bodies, diameter, width, L, a*, b*). Larger circles indicate the average position of a cluster's data.

3.2. Qualitative Traits

Lighting wavelength did not affect qualitative traits. The mushroom pH was not affected by substrate (Table 4). The substrate had a significant effect on electric conductivity, total soluble solids, and titratable acidity. Mushrooms grown on S1, however, were characterized by lower values (EC of 4.34 mS cm^{-1}, 2.91 °Brix, and 0.72% of citric acid equivalent).

Table 4. Effect of substrate and lighting treatments on pH, electric conductivity, total soluble solids, and titratable acidity of *Pleurotus ostreatus* fruiting bodies. Means are followed by standard error. Within each parameter, values without letters in common differ at p-value ≤ 0.05 according to Tukey's HSD test.

	pH	**EC (mS cm^{-1})**	**Total Soluble Solids (°Brix)**	**Titratable Acidity (% Citric Acid eq.)**
		Substrate		
S1	6.34 ± 0.04	4.34 ± 0.34 b	2.91 ± 0.24 b	0.72 ± 0.09 c
S2	6.26 ± 0.04	5.24 ± 0.13 a	3.78 ± 0.22 a	1.30 ± 0.07 a
S3	6.30 ± 0.05	4.78 ± 0.17 ab	3.24 ± 0.19 ab	1.00 ± 0.06 b
p-value	*ns*	*0.0345*	*0.0165*	*0.000*
		Light treatments		
Control	6.24 ± 0.04	4.44 ± 0.33	2.97 ± 0.22	1.01 ± 0.11
Red	6.28 ± 0.06	5.17 ± 0.16	3.71 ± 0.24	1.06 ± 0.15
Red + Blue	6.35 ± 0.03	4.95 ± 0.14	3.52 ± 0.25	1.03 ± 0.04
Blue	6.32 ± 0.06	4.63 ± 0.41	3.05 ± 0.34	0.96 ± 0.15
p-value	*ns*	*ns*	*ns*	*ns*

Within the qualitative parameters, the concentration of vitamin D_2 was strongly influenced by the light treatment (Figure 5). B and R + B light increased the amount of vitamin D_2 by +38.8% and +34.3%, respectively, compared to control (59 µg kg^{-1}dw), with a total amount of 93 and 90 µg kg^{-1} dw. Conversely, R light had a negative effect on vitamin

D_2 biosynthesis, reducing it by 11% compared to control. Substrate treatments did not affect vitamin D_2 content, as shown in Figure 5, with S1 substrate obtaining 83 µg kg^{-1}dw, S2 63 µg kg^{-1}dw, and S3 75 µg kg^{-1}dw.

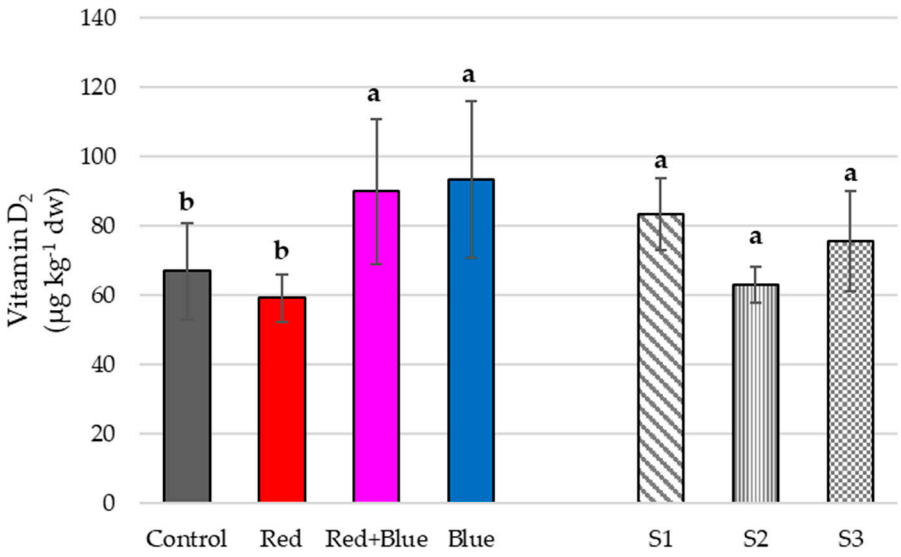


Figure 5. Effect of light wavelength and substrate on vitamin D_2 content in *P. ostreatus*. Values without common letters significantly differ at p-value ≤ 0.05 according to Tukey's HSD test. Bars indicate standard error.

4. Discussion

4.1. Productive Traits

4.1.1. Lighting Treatments

The results of this experiment demonstrate that the utilization of lighting treatments had a noticeable impact on specific productive traits. The development of primordia occurred quicker when exposed to R light. A similar phenomenon was observed by Roshita and Goh in 2018 with *P. sajor caju* and *P. florida*, indicating that under different light treatments, primordia developed more rapidly compared with standard light conditions [28]. In this case, it was specifically the R light that promoted primordia formation [24]. Yield, on the other hand, remained unaffected by light treatment. There are limited studies in the literature concerning the impact of light treatments on quantitative production, and these studies provided contradictory results, even for the same species., Yue et al. [21] found that red and far-red lights had a positive influence on *P. eryngii* yield [21], whereas Du et al., in 2020, demonstrated that blue light increased the yield of *P. eryngii* more than red light [23]. Additionally, results among species within the same genus vary. Although all light treatments were effective in enhancing yield, the yield of *P. sajor-caju* was lower than that of *P. florida*; the blue light had the biggest impact on yield in both species, as noted by Roshita and Goh in 2018 [28]. Most experiments involving artificial light treatments used small transparent plastic bags or transparent bottles to observe the effects throughout the entire growth cycle. In contrast, most of the bags in this experiment were white, and artificial light treatments were only able to impact mushroom development from primordia appearance to the full growth of fruiting bodies. Moreover, to provide a more comprehensive understanding of the yield results, it is necessary to consider other productive characteristics, such as the number of fruiting bodies for each cluster, which was highest when grown under control lighting, R, and R+B light, whereas the B light was the only treatment that negatively impacted this number. Typically, the primary features for characterizing clusters include the number of fruiting bodies and the average diameter and width of the cap. In this experiment, the number of fruiting bodies showed a slight increase under R light exposure. Furthermore, light treatments affected the size of the caps, especially B

light, which increased the average diameter of caps compared to control lighting. Similar results were reported by Yue et al. for *P. eryngii* [21]. B light had a pronounced effect on cap morphology, which may also influence the weight of mushrooms. In this experiment, light treatments with a lower percentage of B light, as in the R+B light, obtained similar results. Wang et al., in 2020, discovered that in *P. ostreatus*, blue light upregulates genes in the pileus involved in glycolysis and the pentose phosphate pathway, which are essential pathways for ATP production and pileus growth. However, this regulation differs in the stipe and gill, where these genes are unaffected by B light, and for this reason, width of fruiting bodies remained unaffected by lighting treatments, unlike the diameter [22]. Additionally, the color of the cap was influenced by light, which supports the findings of Du et al., who observed that B light in *P. eryngii* could darken the cap color. Du et al. reported that some genes related to tyrosinase, which is closely associated with melanogenesis, were upregulated by B light [23]. In this study, all lighting treatments containing B light resulted in a darker cap colour with a lower L* value. Roshita also observed a similar effect with *P. sajor-caju*, which exhibited a darker color under green, B, and R lights compared to control [28]. Moreover, in this study, the a* color parameter was also significantly different, indicating the presence of greener tones under B light compared to control lighting.

4.1.2. Substrate

Substrate treatments had an impact on several production characteristics, particularly the formation and number of primordia. Each substrate used in this study was composed of the same raw materials but featured different nitrogen amounts, according to the company's proprietary protocols. The chemical composition of all substrates exhibited slight variations in terms of total nitrogen content and the C/N ratio; both parameters are potentially relevant to yield outcomes. Notably, in substrates S1 and S2, the primordia production started at 23 DAI, according to the findings of Muswati et al., who reported a 30-day period for primordia formation in *P. ostreatus* cultivated in only straw matrix [29]. In our study, the addition of nitrogen supplementation likely facilitated faster mycelium development, thus promoting earlier primordia initiation. The number of primordia appeared to be correlated with the number of clusters per kilogram of substrate, and the substrate S1 displayed a higher value of both primordia and clusters. While yield differences were not statistically significant, there were relevant differences in mushroom morphology. The substrate S3 had the lowest number of clusters but the largest diameter among fruiting bodies, a phenomenon also noted by Hoa et al. [30]. It is well established that the substrate can have a significant impact on the morphological characteristics of fruiting bodies. However, the average number of fruiting bodies per cluster and the width of fruiting bodies did not exhibit any significant differences across the various substrate treatments. The color of the cap was influenced by the industrial substrate and resulted in higher L* values, indicating a lighter color as well as higher a* values, signifying a greener hue, in substrates S1 and S2 compared to substrate S3. This is consistent with the findings of Marino et al. (2003), who emphasized that cap coloration can be primarily influenced by light [31], and in our study, different types of substrates also influenced color. This research revealed that even substrates composed of the same materials but supplemented industrially exhibit noteworthy differences. These disparities warrant further in-depth investigation to better understand their implications.

4.2. Qualitative Traits

The pH, EC, total soluble solids, and titratable acidity content were not influenced by the lighting treatments applied during the cultivation period. However, the substrate had an impact on the qualitative characteristics of oyster mushroom fruiting bodies. The mushrooms harvested from substrate S2 exhibited higher values for EC, total soluble solids, and citric acid content. It is important to underline that the average data obtained in this study differ significantly from those commonly found in the literature. A study by Villaescusa and Gil reported values of 5.1 °Brix and 0.10% citric acid content [32], but it is

worth emphasizing that these qualitative characteristics are primarily influenced by the mushroom strain and the specific substrate used [33].

Vitamin D_2

One significant finding in this study is the impact of light treatments on the nutritional characteristics of *P. ostreatus*. As reported by Cardwell et al., edible mushrooms are known to be a rich source of vitamin D_2 due to their high ergosterol concentration in cell walls, which serves as a precursor for ergocalciferol (vitamin D_2) [19]. Typically, edible mushrooms are fortified with UV light during post-harvest treatment [16,19,33]. However, this study reveals that it is possible to enhance the concentration of vitamin D_2 through LED exposure in pre-harvest treatments, specifically by utilizing wavelengths that are close to UV, such as B light (500–450 nm). This effect was observed in both B light and R+B light treatments, indicating that even a lower percentage of B light in the spectrum can influence vitamin D_2 biosynthesis. The European Food Safety Authority (EFSA) recommends a daily intake of 15 µg of vitamin D_2. However, this recommended value is often not met, particularly in certain geographic areas, such as those at high latitudes. In the European Union (EU), the estimated average dietary intake of vitamin D_2 is calculated to be 2–4 µg day^{-1}, while in the United States, it is 5–6 µg day^{-1}. To address this issue, the introduction of biofortified foods, such as mushrooms with elevated vitamin D_2 levels, could contribute to enhancing daily intake. For instance, 100 g of fresh mushrooms cultivated under B light conditions could result in the assimilation of 6.2% daily vitamin D_2 intake, with a value of 0.93 µg 100 g^{-1} fresh weight [19]. Ložnjak et al. reported that the amount of vitamin D_2 was not influenced by cooking methods; for instance, after 90 min of boiling 62% of vitamin D_2 remains in the product, whereas after pan-fried cooking, 88–81% persists in the fruiting bodies [34]. The implementation of biofortification techniques during mushroom cultivation to enhance the vitamin D_2 content represents an innovative approach to augmenting the nutritional value of mushrooms. Consequently, such advancements hold the potential to elevate the market value of this crop for farmers. However, it is essential to acknowledge that the application of light throughout the cultivation process, particularly during two flushes of *P. ostreatus*, may incur additional production costs. Specifically, incorporating a photoperiod of 8 h and radiation intensity ranging between 30 and 50 µmol m^{-2} s^{-1} can lead to a production cost increase of approximately 12%, equivalent to 3.5 € m^{-2}, with an average energy cost of 0.27 € kW^{-1} (Europe average value). Despite the associated rise in production expenses, it is plausible that consumers may be willing to pay a premium for biofortified foods, provided they are well informed about the associated health benefits. This assertion aligns with the findings of Timpanaro et al., who observed a heightened consumer willingness to pay a higher price for biofortified products. Thus, the potential for increased production costs could be offset by the perceived value attributed to the enhanced nutritional content of biofortified mushrooms [35].

5. Conclusions

In conclusion, the application of LED lighting at specific wavelengths exerted a significant influence on mycelia development, warranting further investigation in subsequent studies. While various light sources do not notably enhance overall yield, they play an important role in shaping the morphological characteristics of fruiting bodies. For instance, B light has a particularly pronounced impact on the diameter of fruiting bodies, a trait of considerable importance to consumers and the market. Additionally, both B and R+B lights enhance the vitamin D_2 content during cultivation, thereby potentially increasing the nutritional value of mushrooms. The examination of straw-based substrates revealed that despite yielding similar overall quantities, variations in supplementation and substrate preparation treatments can affect certain productive characteristics, such as the formation of primordia and cap diameter.

Further investigations are imperative to refine the application of different wavelengths during the cultivation period of *P. ostreatus* and to delineate their effects on mycelial devel-

opment during the incubation phase. Moreover, the intensity of radiation and photoperiod necessitate ongoing scrutiny to deepen our understanding of this technique's application. A better understanding of how light intensity, wavelength, and photoperiod affect mushroom yield and nutrition could improve the demand and, subsequently, the retail price of mushrooms, giving growers the information needed to determine the economics of incorporating an LED lighting system into their operation.

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