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The Effect of LED Lighting Durations on Growth, Yield and Quality of Mustard (*Brassica juncea* L.) under Greenhouse Condition

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Abstract

Light-emitting diodes (LEDs) show promise as a radiation source for intensive crop cultivation systems, especially for vegetables in the greenhouse. Regarding mustard greens, most studies have investigated the effects of LED lights at the seedling stage under chamber conditions for microgreens not addressed throughout their growing period up to harvest. Therefore, this study is focused to evaluate the effect of additional LED lighting time from 6 pm on the growth, yield and quality of mustard greens under a greenhouse in Kien Giang province as a typical tropical region. The experiment was arranged in a completely randomized design with 3 replications, from August 1st to September 18th, 2023. Four treatments: AL0, AL2, AL4 and AL6 correspond to lighting durations of 0, 2, 4 and 6 h of LED light, which includes a combination of 3 white lights: 1 pink light, 58 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD, and a Blue (18 %): Green (41 %): Red (41 %) ratio. Results showed that AL4 had the most significant positive impact on mustard plant growth, with higher dry and fresh weight, plant height, leaf width and length. However, AL6 inhibited most parameters except leaf number. Additionally, under AL4 conditions, mustard allocated more biomass to leaves, while those under AL6 conditions allocated more biomass to stems. In terms of quality, LED lighting duration negatively influenced protein content. For instance, AL0 showed the significantly greatest protein value (2.87 ± 0.08 $g \cdot kg^{-1}$) compared to others. Apart from AL0, AL6 had a considerably higher value of protein content (2.58 ± 0.2 $g \cdot kg^{-1}$) compared to AL2 (1.74 ± 0.04 $g \cdot kg^{-1}$) and AL4 (2.17 ± 0.07 $g \cdot kg^{-1}$). The additional durations of LED lighting influenced mature mustard greens' morphology, physiology and quality, providing valuable scientific information for improving vegetable quality and quantity in tropical zones.

Keywords: Biomass, Yield, Artificial lighting, Extended photoperiods

Introduction

The Vietnamese Mekong Delta (VMD), Vietnam's largest aquaculture and agriculture production area, has been known as markedly susceptible to the impacts of climate change [1]. The growth and development of crops are affected by many factors, such as varieties, soil, water and climate. Climate change causes weather and climate to follow a reasonably clear rule and become more unpredictable. Such as floods, droughts, natural disasters and saltwater intrusion occur more frequently. These directly impact crop growth and development - the conditions to ensure crop yield [2]. To minimize weather's adverse effects, greenhouse and indoor cultivation promise growers a controlled growing environment. However, supplemental lighting is a consideration for these farming systems to help better control the photoperiod [3].

Mustard Greens (*Brassica juncea* L.) Czern) is the most commonly consumed vegetable in large volumes [4]. Mustard green could be grown well at temperatures of 15 to 29 °C; however, it will be harmed if the temperature drops below 10 °C or above 32 °C [5]. The availability of optimum light is one of the essential environmental resources for the plant's growth, productivity and survival. Variation in light intensities leads to the development of acclimation strategies in plants in the given environment [6]. According to a study by Alam *et al.* [7], carried out in mustard in a semi-arid agroclimatic region, showed that mustard demonstrated its low-light adaptive trends by slowing down the rates of net CO_2 assimilation, stomatal conductance, transpiration, thylakoid electron transport rate and leaf wax level. Photochemical results showed a critical effect by decreased photosystem II quantum yield, photochemical quenching, and higher non-photochemical quenching.

With the advancement of science and technology, the use of LED lighting (light-emitting diode) has allowed scientists to study not only photosynthetic photon flux density (PPFD) at the plant level but also the relative spectral photon flux density (RSPD) distribution of light. The influence of spectral photon flux density on plant growth and morphology has been studied using several types of LEDs and plant species [8].

Many studies have assessed the effects of supplemental light irradiation, intensity and duration on strawberries [9], watermelon [10], crassulaceae [11], lettuce [12-16], tomato [17,18], cucumber [19], *Sinningia speciosa* [20]. The results of these studies show that supplemental lighting, intensity and photoperiod using artificial light have contributed greatly to plant growth and development. They indicate that increasing the duration of lighting could promote the dry weight of cumulative biomass.

Therefore, the objective of this study was to evaluate the effects of various extended photoperiods after Sunset based on the irradiation of LED lamps on the growth, yield and quality of mustard greens (*Brassica juncea* L.).

Materials and methods

The experiment was conducted from August 1 to September 18, 2023 in a greenhouse with a height of 4 m at the experimental garden of Kien Giang University, Chau Thanh district, Kien Giang province, Vietnam (105°14'33.21" E; 9°91'41.13" N). This period is during the rainy season, so light levels are often lower compared to the dry season. The plants were grown in a greenhouse with environmental conditions such as humidity, temperature and light intensity, as presented in **Table 2**.

Treatments, design and experimental procedures

Four supplementary lighting treatments that included were arranged in a completely randomized design with 3 replications (12 plots in total). The area of each plot was equal to 2 m^2. The spectral distribution of light was measured using a spectroradiometer (OHSP-350 BF). The illuminance (Lux) and photosynthetic photon flux density (PPFD) were measured using a Photosynthesis Light Quantum Meter (TES 1339P). The air humidity and temperature were measured using an Indoor Digital Temperature Humidity Meter (Beurer, Type: HM 16, Art.-Nr: 679.15).

Mustard green seeds were purchased from Dai Dia Seeds Ltd. (Dist. 6, Ho Chi Minh City, Vietnam). Seeds were treated by soaking in 3 boiling and 2 cold water for 2 h and incubated in dark conditions. When the seeds germinate, they are sown on Tribat soil at a 0.2 g/m^2 seeding density. Tribat soil contains the following nutrients: Humus (15%); organic matter (25%); total nitrogen (1%), potassium K_2O: 0.73%, total phosphate (P_2O_5: 0.3%), cation exchange capacity (CEC: 45meq/100g) and other nutrients (contents were not clarified by the company) such as Mg, Mn, Zn, B, Cu, Mo and Chelate-iron.

When the plant has the third leaf appear, illumination was supplied by combined irradiators based on LED modules of the same photosynthesis photon flux density at various extending photoperiods. The LED module was combined from 4 CoB LEDs (1 pink light at the center and 3 white lights installed around). All the CoB LEDs have the exact specifications, such as Voltage: 12 - 14 V DC; Power: 50 W; Brightness: 90 - 120 LM/W; Color Rendering Index (CRI): 60 - 85; Beam Angle: 120 °; Operating Temperature: −20 to 60 °C; Color Temperature: 4,500 K; spectral bandwidth: 400 - 780 nm. LED modules were adjusted to keep 0.5 m from the tip of the leaves. For the control treatment, mustard green was only exposed to natural light during the day. For the rest of the treatments, all LED modules were controlled independently by custom-made time to turn on and off the LED lamps with digital controls (**Table 1**).

Table 1 Various extending photoperiod regimes using LED module.

Irradiation treatment	Light on	Light off
A0 (Control)	-	-
A2	6 pm	8 pm
A4	6 pm	10 pm
A6	6 pm	12 pm

Growth and yield components

The first measurement was performed when the plants had 3 full leaves, and then at weekly intervals. Ten plants in each plot were randomly selected and marked to collect growth data. Leaf length (from the tip of the longest leaf to the petiole), leaf width (widest point) and height of plants (from the ground level to the tip of the topmost leaf) were measured.

Cutting all above-ground parts collected the yield components 45 days after seeding. Leaves and stems were separated and weighed to calculate fresh yield. Then, they were oven-dried at 65 °C for 72 h until a constant weight was reached to calculate dry matter.

Nutritional quality components

The oven-drying method determined water content (%) at 105 °C. Lipid content (%) was determined according to the indirect method suggested by So and Thuan [21]. Vitamin C ($g \cdot kg^{-1}$) was determined by the spectrophotometric method [22]. Protein ($g \cdot kg^{-1}$) was determined according to the Lowry method [23]. Nitrate ($mg \cdot kg^{-1}$) was determined by standard colorimetric procedures [24].

Statistical analysis

Data were analyzed using the SPSS statistical package (version 20). One-way ANOVA with 4 treatments was performed using Duncan's test at $p \leq 0.05$.

Results and discussion

The result showed that, values of light intensity and PPFD in the greenhouse were 2,159 ± 216 - 9,380 ± 482 (lx) and 44.51 ± 5.74 - 156.65 ± 9.40 (μmol·m^{-2}·s^{-1}) (**Table 2**), respectively. When compared to conditions outside the greenhouse, indicators inside the greenhouse directly related to photosynthesis, such as light intensity and PPFD, decreased by 87.25 and 87.17 %, respectively. These decreases could be attributed to transparancy film of greenhouse proof and sunshade net. The LED light values of LUX, CF and PPFD and the RED, Green and Blue ratios at night time are presented in **Figures 1** and **2**, respectively. The spectra, intensity and photoperiod of illumination play an essential role in cultivating plants under greenhouse conditions, especially for vegetables. LEDs show features of narrow spectral bands, but intensity and time regimes are regulated within a wide range, making them a potential basis for using a combination of LEDs for plant cultivation. LED light is the energy source used by plants and regulates photosynthesis and other physiological processes [25]. Therefore, precise management of irradiance and wavelength may hold promise in maximizing the economic efficiency of plant production, quality and nutritional potential of vegetables grown in controlled environments [12]. All supplemental treatments of LED had varying impacts on morphology that depended on crop age. [26].

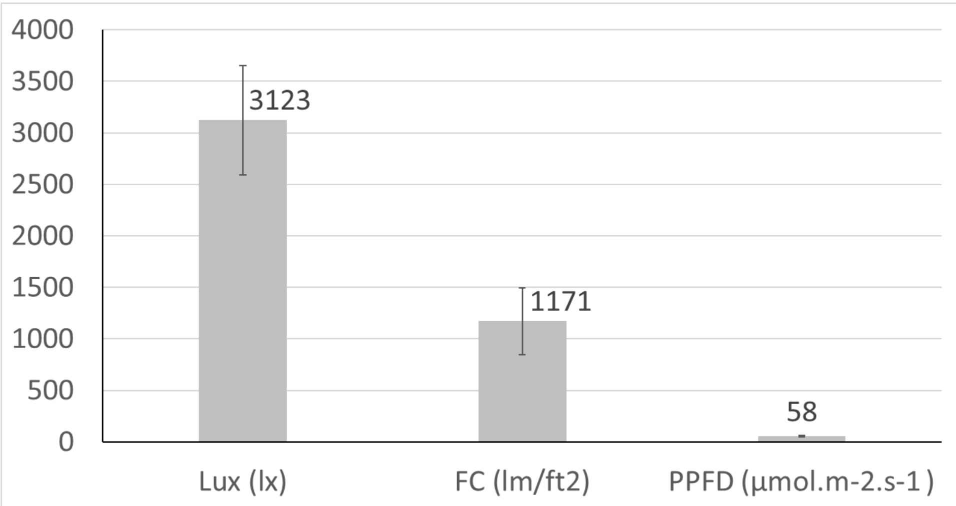

Figure 1 Lux, FC and PPFD value under LED at night time.

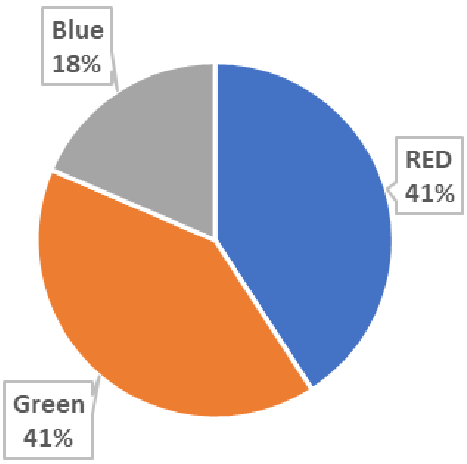

Figure 2 Red, Green and Blue ratio of LED.

Table 2 Air humidity, temperature, lux and PPFD means of daytime in the greenhouse condition.

Mean	Humidity (%)	Temperature (°C)	Lux (lx)	PPFD ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
Max	60.22 ± 1.38	34.02 ± 0.50	9,380 ± 482	156.65 ± 9.40
Min	73.06 ± 1.04	29.69 ± 0.54	2,159 ± 216	44.51 ± 5.74

Note: The mean of the maximum or minimum values was averaged from all daily maximum or minimum values during the experimental time. The symbol "±" indicates standard error.

Effect of extended illumination duration on growth and development of mustard greens *(Brassica juncea* L.*)* on tribal condition

Plant height

The result showed that the LED lights after 14 days of additional lighting was effective (i.e., day 28 DAS), where the height plant in AL4 reached the highest statistically significant value compared to the AL0 and AL6. However, plant height in AL4 and AL6 was similar, and AL6 reached the highest value, a statistically significant difference compared to AL0 and AL2 at 35 DAS and a statistically significant difference with others at stage 42 DAS (**Table 3**). This shows that the additional 6 h of LED lighting is unsuitable for plant height growth at the 28 DAS stage, but the height of plants in AL6 grew better than other treatments later. The effects of the lighting treatments on plant height at flowering varied among species of *Sinningia speciosa* [20] however, positive effects on height plants were demonstrated, such as on strawberry [9], lettuce [16], on Joseph's coat, 'Brazilian Red Hots' [27] because LED light is the energy source that is used by plants as well as the regulator of photosynthesis and the regulator of other physiological processes [25]. Our results showed that LED lights positively affected the height of mustard plants. However, the height of the mustard plant corresponded to the earlier stage of 4-hour light than 6 h. Lighting duration longer than 4 h can lead to physiological disturbances in the plant and which could be attributed to the fact that mustard gets stressed at an earlier stage under a more extended photoperiod of AL6 due to increasing H_2O_2 and the antioxidant enzyme activities, respectively [10].

Table 3 Effect of LED lighting time on plant height (cm) of mustard greens during growing stages.

Treatment	Mean plant height (cm)				
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS
AL0	15.02 ± 0.15	22.36 ± 0.69	25.98 ± 0.71^{b}	31.03 ± 0.85^{b}	34.39 ± 1.29^{b}
AL2	15.27 ± 0.23	24.39 ± 0.52	28.42 ± 1.00^{ab}	33.20 ± 1.41^{b}	36.72 ± 1.50^{b}
AL4	15.48 ± 0.28	23.67 ± 0.61	30.87 ± 1.47^{a}	37.73 ± 1.36a^{b}	44.33 ± 1.77^{b}
AL6	15.52 ± 0.23	22.23 ± 0.90	26.38 ± 1.10^{b}	40.11 ± 4.38^{a}	60.11 ± 5.67^{a}
Sig.	Ns	ns	**	*	***

Marginal means of a column not sharing any letter within plant height is significantly different by the Duncan test at the 5 % (*), 1 % (**), 1 ‰ (***) levels and non-significance (ns).

Leaf features

Leaf length

Leaf length appeared quite sensitive to the duration of additional LED. However, 2 distinct trends were evident. There was a statistically significant difference in leaf length between treatments immediately after 1 week of additional LED lighting. Treatment AL6 had a significantly shorter leaf length (14.48 ± 0.76 cm) than treatment AL0 (17.21 ± 0.43 cm). Conversely, after 2 weeks of lighting (28 DAS), treatment AL4 exhibited the highest leaf length, with a statistically significant difference from all other treatments, a trend that persisted until 42 DAS. Notably, the AL6 treatment showed the lowest length. The overall results indicate that, the duration of additional LED lighting impacts leaf length, with 4 h of exposure yielding the best results, while 6 h of exposure inhibited the length of green mustard leaves (**Table 4**).

Table 4 Effect of LED lighting time on leaf length (cm) of mustard greens during growing stages.

Treatment	Leaf length (cm)				
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS
AL0	10.06 ± 0.33	17.21 ± 0.43^{a}	19.63 ± 0.65^{b}	22.33 ± 0.80^{b}	24.39 ± 0.95^{b}
AL2	10.57 ± 0.66	16.36 ± 0.57^{ab}	17.98 ± 0.55^{bc}	20.23 ± 0.90^{bc}	22.61 ± 1.31^{b}
AL4	10.68 ± 0.51	16.39 ± 1.21^{ab}	22.50 ± 1.30^{a}	25.51 ± 0.65^{a}	28.02 ± 0.68^{a}
AL6	10.06 ± 0.48	14.48 ± 0.76^{b}	16.83 ± 0.61^{c}	18.06 ± 0.50^{c}	19.27 ± 0.54^{c}
Sig.	Ns	*	***	***	***

Marginal means of a column not sharing any letter within leaf length is significantly different by the Duncan test at the 5 % (*), 1 ‰ (***) levels and non-significance (ns).

Leaf width

Compared to leaf length, the effect of the timing of adding light on leaf width tends was the same after 3 weeks of lighting. AL4 showed the best width, with a statistically significant difference compared to all treatments at 35 DAS (8.87 ± 0.29 cm) and 42 DAS (10.02 ± 0.40 cm), especially the effect of AL6 on leaf width was opposite, with the lowest leaf width value (**Table 5**). Notably, there was a remarkable difference in leaf width at earlier stage (21 DAS), in which both AL4 (5.07 ± 0.38 cm) and AL6 (4.83 ± 0.35 cm) are statistically significantly lower than the AL0 treatment (6.03 ± 0.19 cm); later on leaf width at AL4 increased and reaching the highest value at stages 35 and 42 DAS. This shows that mustard greens had adapted to the additional LED light after 3 weeks of exposure, explicitly changing from adverse effects at the 21 DAS stage to positive effects under AL4 conditions afterward, but longer duration (AL6) maintained the trend with the lowest values.

Table 5 Effect of LED lighting time on leaf width (cm) of mustard greens during growing stages.

Treatment	Leaf width (cm)				
	14 DAS	21 DAS	28 DAS	35 DAS	42 DAS
AL0	3.14 ± 0.02	6.03 ± 0.19^{a}	7.00 ± 0.25^{ab}	7.80 ± 0.30^{b}	8.47 ± 0.35^{b}
AL2	3.16 ± 0.05	5.47 ± 0.18^{ab}	6.16 ± 0.18^{bc}	7.04 ± 0.35^{bc}	7.54 ± 0.42^{bc}
AL4	3.17 ± 0.05	5.07 ± 0.38^{b}	7.30 ± 0.49^{a}	8.87 ± 0.29^{a}	10.02 ± 0.40^{a}
AL6	3.17 ± 0.05	4.83 ± 0.35^{b}	5.58 ± 0.36^{c}	6.03 ± 0.35^{c}	6.63 ± 0.36^{c}
Sig.	Ns	*	**	***	***

Marginal means of a column not sharing any letter within leaf width is significantly different by the Duncan test at the 5 % (*), 1 % (**), 1 ‰ (***) levels and non-significance (ns).

In our experiment, we found that increasing the duration of additional lighting caused mustard leaf length and width changes. Mustard plants subjected to 4 - 6 h of additional LED lighting had smaller values of leaf length and width at 21 DAS, but 4 h of additional LED lighting had greater values at 35 DAS, while 6 h of additional LED lighting still kept the lowest values afterward. This shows that adding LED lighting after 6 pm for a duration of 4 h is the best condition to increase leaf length and width. The purpose of our experiment was to add LED lights to prolong the photosynthesis time of mustard greens because the positive effect of LED lights on the growth of mustard greens has been documented by many studies due to enhanced plant photosynthesis. There was a positive correlation between leaf width and leaf length with leaf area [26], meaning that any factor influencing leaf area could also result in changes in leaf width and length. Previous research has shown that the influence of LED lighting on leaf area varies depending on the plant species. For example, it was 2.7 times greater in strawberries due to the acceleration of leaf photosynthesis resulting from higher light intensity on the leaf surface [9], and the blue-red spectrum condition is the best lighting option for growing red mustard with a larger leaf area (60 %) [28]. This is in contrast to research on lettuce under Red Blue lighting [12]. Another study showed that the influence of LED lighting on lettuce leaf length and width depended on the combination of light/dark ratio and LED type [16] and all supplemental treatments of LED had varying impacts on lettuce morphology that were dependent on crop age [26].

Number of leaves

A significant difference in the number of leaves due to the lengthened lighting LED was only observed at the final stages (35 and 42 DAS); specifically, the AL6 had the highest number of leaves, which was statistically significant compared to AL0 at 35 DAS and AL2 at 42 DAS. Similar to the height growth rate, the number of green mustard leaves in AL6 was the highest in period 3 (**Table 6**). Based on observation, green mustard plants in AL6 was switching from vegetative to flowering only. Effects of LED on flowering of plants were observed in the previous paper [27,28]. This indicates that LED lights impact the number of leaves of mustard plants, but it is more pronounced at a later stage in the growth process. Our result is consistent with previous research that showed that the leaf number of lettuce was high under 2 purple LEDs combined with a light/dark ratio of 18/6 [16] and LED with WFR (White Far Red) and RGB (Red Green Blue) also increased the leaf number of lettuce [26].

Table 6 Effect of LED lighting time on leaf number of mustard greens during growing stages.

Treatment	Leaf number				
	14 DAS	**21 DAS**	**28 DAS**	**35 DAS**	**42 DAS**
AL0	3.00 ± 0.00	4.22 ± 0.15	5.33 ± 0.17	6.00 ± 0.00^{b}	6.00 ± 0.00^{b}
AL2	3.11 ± 0.11	4.44 ± 0.18	5.22 ± 0.15	6.00 ± 0.00^{b}	6.33 ± 0.17^{ab}
AL4	3.11 ± 0.11	4.44 ± 0.18	5.44 ± 0.18	6.33 ± 0.17^{ab}	6.78 ± 0.15^{a}
AL6	3.00 ± 0.00	4.33 ± 0.17	5.00 ± 0.00	6.44 ± 0.18^{a}	6.67 ± 0.17^{a}
Sig.	ns	ns	ns	**	**

Marginal means of a column not sharing any letter within leaf number is significantly different by the Duncan test at the 1 % (**), levels and non-significance (ns).

Yield components

Fresh and dry biomass consistently trended throughout the observation (**Table 7**). AL4 consistently had a statistically significant advantage over the other treatments in most indicators, except for stem dry biomass. An interesting observation was that AL6 resulted in significantly lower fresh leaf weight, fresh shoot weight and dry shoot weight than AL0. Additionally, the additional 2 h of lighting (AL2) did not significantly affect the tested parameters. This indicates that LED lighting impacts both the dry and fresh biomass of mustard plant parts, with AL4 showing a positive effect on biomass while AL6 had a negative effect. It seems that AL2 does not sufficiently impact biomass indicators, so no significant difference in fresh and dry biomass of all parts of the plant was found. The increase in leaf photosynthesis is closely associated with similar increases in crop yield or biomass [29]. LED light serves as the energy source for plants and regulates photosynthesis and other physiological processes [15], allowing wavelength to be matched to plant photoreceptors to provide more optimal production and to influence plant morphology and composition [30]. Different plant species and parts respond differently to LED factors, depending on factors such as negative effect on biomass under low light conditions [31], a positive relationship between light intensity in the biomass and photosynthetic parameters [32], light-quality treatments, with the best growth observed under Red Blue White light [12], RED light [33], Red-Blue LED light enhances the growth and productivity [34], photosynthesis photon flux density (PPFD) of 107 $\mu mol \cdot m^{-2} \cdot s^{-1}$ [28]; light duration [16,17]. Our study on mustard under different conditions yielded consistent results with previous findings, particularly regarding lighting duration combined with PPFD. For example, AL4 (16 h of light including 12 h of sunlight and 4 h of LED with 44.51 ± 5.74 - 156.65 ± 9.40 PPFD and 58 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD) showed the best dry and fresh biomass. However, the results were reversed when we increased the light duration to 18 h.

Table 7 Effect of LED lighting time on yield components of mustard greens.

Treatment	Weight at harvest time (g/plant)							
	Fresh leaves	**Fresh stem**	**Fresh root**	**Fresh shoot**	**Dry leaves**	**Dry stem**	**Dry root**	**Dry shoot**
AL0	11.11 ± 0.26^{b}	3.68 ± 0.17^{b}	3.04 ± 0.17^{b}	14.80 ± 0.33^{b}	0.54 ± 0.04^{bc}	0.34 ± 0.02	0.03 ± 0.00^{b}	0.89 ± 0.04^{b}
AL2	10.51 ± 0.29^{b}	3.94 ± 0.18^{b}	3.36 ± 0.16^{b}	14.46 ± 0.35^{bc}	0.57 ± 0.05^{b}	0.34 ± 0.02	0.04 ± 0.00^{b}	0.92 ± 0.05^{b}
AL4	14.44 ± 0.63^{a}	4.71 ± 0.18^{a}	4.33 ± 0.24^{a}	19.16 ± 0.61^{a}	0.73 ± 0.05^{a}	0.35 ± 0.02	0.05 ± 0.00^{a}	1.08 ± 0.06^{a}
AL6	8.70 ± 0.76^{c}	4.14 ± 0.34^{ab}	2.89 ± 0.11^{b}	12.84 ± 0.81^{c}	0.42 ± 0.02^{c}	0.30 ± 0.02	0.03 ± 0.00^{b}	0.71 ± 0.02^{c}
Sig.	***	*	***	***	***	ns	***	***

Marginal means of a column not sharing any letter within components of shoot weight are significantly different by the Duncan test at the 5 % (*), 1 ‰ (***) levels and non-significance (ns).

Understanding how plants partition their new biomass is crucial for studying competition between crops and weeds [35]. Previous research has explored plant partitioning, focusing on allocating biomass to above and below-ground parts. Shoots are responsible for light harvesting and energy production, while roots are vital in nutrient and water uptake. Plants with more extensive root systems tend to have higher biomass and root-to-shoot ratios [36]. Various factors influence biomass partitioning in plants. For example, low light conditions can significantly affect the biomass allocation, impacting root, shoot, root-to-shoot ratio and total plant dry biomass [31] drought conditions [37], different light spectra [38] e.g., red and blue light treatments [12] and high temperatures [39], incredibly different sowing dates and growing environments [40-42]. **Table 8** displays the biomass allocation under different LED lighting conditions in our experiment results. The treatments had no significant differences in fresh and dry root biomass allocation. However, treatment AL6 showed the highest allocation of fresh stem biomass (26.60 ± 2.05 %) compared to other treatments, while the allocation to fresh leaves was the lowest (54.56 ± 2.59 %), significantly different from AL0 and AL4. The allocation ratio of dry biomass for leaves and stems mirrored the results of fresh biomass allocation. The dry biomass allocation to leaves in AL4 was higher than in AL6, but the opposite was true for stem dry matter allocation. These findings suggest that the additional LED lighting time does not correlate with the fresh and dry biomass allocation ratio for different plant parts. Still, specific lighting durations have a clear impact. Plants receiving 6 h of additional LED lighting had lower fresh biomass allocation to leaves but higher allocation to stems, while AL4 negatively affected the distribution of dry biomass to stems. In summary, the additional LED lighting time influences the distribution of fresh and dry leaf and stem biomass in mustard plants but not in roots. The biomass distribution to each organ of mustard depends on the duration of additional LED lighting. For example, plants under AL4 conditions allocated more dry and fresh biomass to leaves, while those under AL6 conditions allocated biomass mainly to fresh and dry stems.

Table 8 Effect of LED lighting time on the ratio of biomass allocation of mustard greens.

Treatment	**Biomass partitioning ratio (%)**					
	Fresh root	**Fresh leaves**	**Fresh stem**	**Dry root**	**Dry leaves**	**Dry stem**
AL0	17.04 ± 0.83	62.31 ± 0.96^{a}	20.66 ± 0.84^{bc}	3.55 ± 0.23	58.60 ± 2.30^{ab}	37.84 ± 2.27^{a}
AL2	18.88 ± 0.93	58.99 ± 1.11^{ab}	22.12 ± 0.85^{bc}	3.97 ± 0.34	59.67 ± 2.45^{ab}	36.36 ± 2.19^{a}
AL4	18.51 ± 1.02	61.26 ± 1.32^{a}	20.22 ± 1.10^{c}	4.37 ± 0.42	64.71 ± 1.60^{a}	30.91 ± 1.39^{b}
AL6	18.83 ± 1.32	54.56 ± 2.59^{b}	26.60 ± 2.05^{a}	4.45 ± 0.23	56.26 ± 1.44^{b}	39.29 ± 1.49^{a}
Sig.	ns	*	**	ns	*	*

Quality

Plants grown under LED irradiation showed increased accumulation of primary and secondary metabolites, starch, simple sugars, proteins, vitamin C and phenolic compounds. Previous studies have demonstrated that light quality influences plants' biochemical composition. Remarkably, under the combination of green LEDs (R70:G10:B20) conditions, microgreens of Kohlrabi purple, Cabbage red, Broccoli, Kale Tuscan, Komatsuna red, Tatsoi and Cabbage green, which can benefit human health in conditions with limited food, exhibited the highest growth and nutritional content [43]. For instance, plants grown under Red Blue White (RBW) light had significantly higher soluble sugar content but lower nitrate content than those under Red Blue (RB) light. At the same time, there were no significant differences in the soluble protein content of lettuce leaves among the different light treatments [12].

In a separate study, tomato seedlings exposed to White Blue LED light exhibited a 60.4 % increase in sucrose content compared to those under White LED light [17]. Additionally, photon flux density (PFD) plays a role in influencing plant biochemical. For example, mustard plants exposed to lower PPFD values showed higher nitrate content compared to those exposed to higher PPFD levels [28]. Our results showed a negative correlation between water content and LED lighting duration. In other words, the longer the mustard plant is exposed to light, the lower the water content (**Table 9**). This could be attributed to more water used for photosynthesis and respiration under LED conditions. LEDs demonstrate lower heat emission and higher energy conversion efficiency [30,44]. LED light serves as the energy source for plants and regulates photosynthesis and other physiological processes [15].

Regarding vitamin C content, this parameter was positively correlated with the additional LED lighting time, and there were significant differences among treatments. The vitamin C content of mustard greens in the AL6 treatment was the highest at 9.54 $g \cdot kg^{-1}$, 2.6 times significantly higher than in the AL0 treatment (3.67 ± 0.17 $g \cdot kg^{-1}$). Previous research has shown that higher vitamin C content in plant tissues is associated with higher light intensity [45] and higher PPFD levels (ranging from 110 - 220 µmol $m^{-2} \cdot s^{-1}$) during the growing season [46]. In our experiment, we focused on comparing LED light durations, and our results showed that longer LED lighting durations resulted in higher vitamin C content, which is consistent with previous findings.

Lipid content was significantly higher among AL2 (2.82 ± 0.03 %), AL4 (3.92 ± 0.02 %) and AL6 (3.85 ± 0.01 %) than AL0 (2.10 ± 0.12 %), but no significant difference between AL4 and AL6 was observed (**Table 9**). The results showed that protein and fat were the highest in microgreens under white light. However, supplementation of white light with red or blue light negatively affected the content of protein and fat in Cabbage Microgreens [47]. The color of the LED influenced the fat content. In our experiment, we only observed the impact of lighting duration levels with 1 type of LED (mixed with 3 white lights and 1 red light) with 58 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Our results showed that an additional 4-hour LED lighting is best for fat value, and this value showed a declining trend under a longer duration (additional 6-hour LED lighting).

For protein content, previous studies showed that soluble protein contents of lettuce leaves showed no significant differences among treatments of LED [12]. However, the white light of LED showed a positive impact on the protein of Cabbage Microgreens, while white light with red or blue light had a reversed effect [47], indicating that the impacts of LED on plant protein are still debatable. In our results, our experiment was partially consistent with previous results, e.g., additional light caused a reduction in protein content when compared to AL0 (control) to the rest of the treatment. However, there was a positive relationship between protein content and light duration. AL6 had a 1.2 and 1.5 times higher protein value than AL4 and AL2, respectively. It could be that the protein content lowered at the beginning of the experiment when mustard plants were exposed to additional light treatments (AL2, AL4 and AL6), and they could accumulate protein later as they adapted to the extra light.

Nitrate content in plants depends on the quality of light. For example, nitrate content under the Red Blue treatment was significantly higher than under the Red Blue White treatment [12]. In another study, red mustard grown under low PPFD values (107.0 - 119.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$) showed 13 - 30 % higher nitrate content compared to plants grown under higher PPFD values (160.4 - 178.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$) [28]. Our experiment was conducted under shallow PPFD conditions (58 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to investigate the impact of LED lighting duration levels. Our results showed significant differences among treatments, with the highest nitrate content in AL4 (304.93 $mg \cdot kg^{-1}$), followed by AL2 (302.66 kg^{-1}), AL6 (251.50 kg^{-1}) and AL0 (240.83 kg^{-1}). This indicates that light duration also influences the nitrate content of mustard plants. In summary, LED lighting duration is positively correlated with lipid and vitamin C content but negatively correlated with water content in mustard plants. Adding LED lights for 2 - 4 h increases the NO_3^- content, but this parameter decreases under 6 h of LED light exposure. LED lighting negatively influences protein content, but longer durations of LED exposure result in higher protein content in mustard plants.

Table 9 Effects of lengthened lighting LED on the quality of fresh mustard.

Treatment	Water content (%)	Lipid (%)	Vitamin C ($g \cdot kg^{-1}$)	Protein ($g \cdot kg^{-1}$)	Nitrate ($mg \cdot kg^{-1}$)
AL0	95.81 ± 0.18^a	2.10 ± 0.12^c	3.67 ± 0.17^d	2.87 ± 0.08^a	240.83 ± 0.17^d
AL2	94.28 ± 0.10^b	2.82 ± 0.03^b	5.69 ± 0.09^c	1.74 ± 0.04^d	302.66 ± 1.77^b
AL4	91.72 ± 0.58^c	3.92 ± 0.02^a	5.91 ± 0.01^b	2.17 ± 0.07^c	304.93 ± 0.07^a
AL6	89.36 ± 0.64^d	3.85 ± 0.01^a	9.54 ± 0.10^a	2.58 ± 0.2^b	251.50 ± 1.50^c
Sig.	***	***	***	***	***

Marginal means of a column not sharing any letter within quality parameters of fresh mustard are significantly different by the Duncan test at the 1‰ (***) levels and non-significance (ns).

Conclusions

This study demonstrated that LED lighting at 58 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD after 6 pm with additional lighting durations remarkably influenced parameters such as plant height growth, leaf length, dry and fresh weight, number leaves, quality as well as biomass allocation observed. AL2 did not significantly affect most parameters, while AL4 positively affected dry and fresh weight, plant height, leaf width, and length of mustard plants. AL6 had adverse effects on most parameters except leaf number. Additionally, biomass distribution among different organs varied with the additional lighting duration. Mustard plants under AL4 conditions allocated more biomass to leaves, while those under AL6 conditions allocated more biomass to stems. LED lighting negatively influenced protein content, but longer durations showed higher protein content in mustard plants. There was a positive relationship between the additional lighting duration of LED and lipid content as well as vitamin C but a negative relationship with water content. So an additional 4 h of LED lighting at 58 $\mu mol \cdot m^{-2} \cdot s^{-1}$. PPFD after 6 pm is suitable for the growth of green mustard. The longer the lighting time of LED, the higher the mustard quality.

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