Investigation of led exposure duration and dark phase on the growth rate, yield, and quality of mung bean (*Vigna radiata* L.) microgreens

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Abstract. This study examines the effects of varying LED durations and the seedling's dark phase on microgreen mung bean growth rate, yield, and quality. Conducted in December 2023 at the Laboratory of Microgreen Cultivation, Jl. Joyo Agung, Malang, at an elevation of approximately 625 meters, the study utilized a box experiment with a split-plot design. The primary variable was the length of the LED dark phase (0, 12, and 18 hours), while the subplot focused on the early seedling dark phase treatment (two 24-hour dark phases and no dark phase). Analysis of variance showed no significant interaction between LED exposure time and blackout treatment, except for plant texture. The best plant texture (6.10 mm/s) was achieved with 18 hours of exposure and an early-stage dark period. LED light exposure influenced the total fresh weight, total dry weight, and total soluble solids of the plants. The 12-hour exposure produced the highest total fresh weight (85.15 g), while the 18-hour exposure resulted in the highest total dry weight (6.28 g), crown dry weight (5.70 g), and total soluble solids at 4 and 6 days after sowing (8.80°Brix and 8.27°Brix, respectively).

1 Introduction

In recent years, microgreens have become increasingly popular as a sustainable and nutrient-rich alternative to vegetable crops, particularly in urban farming models [1][2]. This young, tender green vegetable is harvested shortly after germination for its concentrated nutrients and vibrant flavor, making it a promising addition to the modern diet. Therefore, microgreens could be a promising new food source for people who are interested in consuming a healthy diet [3]. Nowadays, microgreens are mainly grown in metropolitan areas because that is where most customers live. Microgreens are an efficient and accessible option for consumers seeking fresh, healthful vegetables in communities with limited space and resources [4]. Their short growth cycle, adaptability to small-scale indoor cultivation, and high nutritional value have made microgreens a key player in the future of urban agriculture as they provide essential vitamins, minerals, and antioxidants. Mung bean (*Vigna radiata* L.)

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microgreens have great potential as a valuable plant-based protein source and offer a more sustainable alternative to traditional protein plants. Mung bean microgreens, rich in proteins and essential amino acids, vitamins, and minerals, can help meet the growing need for nutritious foods in urban and rural areas [5]. Their ease of cultivation, rapid growth cycle, and adaptability to different environments make them attractive options for both small-scale and urban farmers, making them potential candidates for the further development of sustainable food systems.

Several factors must be considered in microgreen cultivation, including temperature, humidity, lighting, and nutrition. Previous research has explored the role of environmental factors, such as light intensity and dark phase, in enhancing the growth and quality of microgreens [6]. It has been experimentally demonstrated that the use of LED lighting has become a preferred method due to its energy efficiency, controllability, and ability to emit specific wavelengths that optimize photosynthesis [7]. The importance of the dark phase in microgreen cultivation was examined in conjunction with LED lighting. The results suggest that periods of darkness can influence plants' metabolic processes and impact their growth rates, yields, and nutritional profiles. We need to expand our understanding of how different LED durations and dark periods may affect microgreens, as most research has been confined to a limited number of species and lighting systems. This study examines the effects of varying LED durations and the seedling's dark phase on the growth rate, yield, and quality of mung bean microgreen.

2 Material and Method

The research was carried out in December 2023-February 2024 at the Microgreen Laboratory of the Faculty of Agriculture, Islamic University of Malang, at an altitude of ± 625 meters above sea level. The microgreens were placed in shaded conditions with a light intensity ranging from 329 to 1181 lux, an average humidity of 40-60%, and a temperature of 25-30°C. The study employed a split-plot design with two factors. The main plots were exposure duration with four levels: 0, 6, 12, and 18 hours of LED exposure. The secondary plots consisted of two levels: dark phase and without dark phase. From these two treatments, eight treatment combinations were obtained. Each treatment was repeated three times with three samples each.

Mung bean seeds were planted in 250 cc planter boxes, weighing 10 g (wet) each. The dark phase treatment was completed within 2x24 hours by stacking the boxes planted with mung bean seeds and placing them in the dark place. Meanwhile, microgreen boxes that did not undergo dark phase treatment were placed in the prepared planting racks. Exposure to LED light was carried out daily according to the treatment from planting to harvest (6 days). Treatment includes 6 hours of radiation per day starting at 7:00 a.m.-1:00 p.m., 12 hours per day starting at 8:00 a.m.-8:00 p.m. and 18 hours starting at 8:00 a.m.-2:00 a.m. The duration of the exposure is set utilizing an electronic timer. Watering is done once a day in the morning using a sprayer tailored to the weather conditions in the planter box. Harvesting occurs when the plants are six days old.

In this study, growth and yield parameters were measured, including relative growth rate (LPR), total fresh weight per box (g), total dry weight per box (g), shoot fresh weight (g), shoot dry weight (g), and the root dry weight (g). LPR is the rate at which plant dry weight increases per unit of time. Each observation included five plant samples. Wet and dry weight of plants were measured on the fourth and sixth days of planting, and the LPR was calculated using the following equation. Meanwhile, the measurement of quality variables includes total dissolved solids (Brix), total vitamin C, total chlorophyll, and texture. Total dissolved solids were measured by smoothing 1 gram of material and adding ten drops of distilled water. The sample was dropped onto the Refractometer device. Measurements were taken 4 and 6 days after planting. Vitamin C measurement is carried out using the iodometric method. To prepare it, 5 grams of the material was flattened, then placed in an Erlenmeyer flask, 5 mL of distilled water was added, and the mixture was then homogenized with a stirrer for 5 minutes. After 5 minutes, the solution was filtered with Whatman paper to separate the filtrate and 1 mL of the extracted solution. The filtered solution was diluted by adding 9 mL of distilled water, and a 5 mL sample was taken and then placed in an Erlenmeyer flask. A total of 0.1 ml or the equivalent of 2 drops of 1% amylum solution was added and then titrated with 0. 01 N-iodine solution until the blue color appeared. Total chlorophyll analysis was performed using SPAD applied to the tip, middle, and base of the leaf. The measurements were taken by clamping the blades on the tool sensor. Chlorophyll observations were made when plants were 4 and 6 days after planting.

3 Result and Discusion

The analysis of variance showed no significant interaction between LED exposure duration and dark phase treatment on the relative growth rate (LPR) of microgreens. The two treatments showed no significant effect independently. Meanwhile, the results of the variance analysis showed that there was also no significant interaction between the combination treatments on the variables of total fresh weight, root fresh weight, and shoot fresh weight. Regardless, the LED exposure duration significantly influenced the total fresh weight of the mung bean microgreens, but no significant influence on the fresh weight of the shoots. The treatment in the dark phase showed no significant influence on the total fresh weight or the fresh weight of the shoots.

The results of the 5% LSD test in Table 1 show that the 12-hour LED exposure treatment produces the highest total fresh weight (42.58 g), which is significantly different from other treatments. However, it was not significantly different from the 18-hour LED exposure treatment. The result of this research is in colinier with the result of [8]. It stated that the growth light accelerated the growth of microgreens as observed from early sprouting, early cotyledon development, and harvest dates compared to the room light. According to Zhang et al. (2020) [9], sufficient light intensity can maximize plant photosynthesis and yield. When light intensity falls below a certain threshold, respiration displaces photosynthesis, and plants become net oxygen consumers. However, photoinhibition can affect photosynthesis and even plant growth as light intensity increases. The study showed that 12 to 18 hours of LED treatment resulted in good fresh weight of plants, although longer lighting decreased fresh weight.

Table 1. Mean of total fresh weight of mung bean microgreen due to LED exposure duration and dark phase treatment

LED treatment	Fresh Weight (g)		
	Total FW	Shoot FW	Root SW
Duration (L)			
0 Hour	37,40 a	30,72	6,68
6 Hours	38,86 ab	32,11	6,74
12 Hours	42,58 c	33,26	9,32
18 Hours	41,06 bc	28,83	12,23
BNT 5%	2,84	tn	tn
Dark Period Treatment(B)			
Without Dark Period	38,25	28,23	10,02
Using dark period	41,70	34,23	7,47
LSD 5%	ns	Ns	ns

Notes : Numbers accompanied by the same letter in the column show no significant difference according to the BNT test at the 5% level, tn = not significant.

The variance analysis results showed no significant interaction on total dry weight and shoot dry weight for the combination of LED exposure treatment and dark phase treatment. Regardless, the treatment of the LED exposure duration significantly influences both variables. The results of the 5% LSD test in Table 2 show that the 18-hour exposure treatment produced microgreens with the highest total dry weight and shoot dry weight (3.14 g and 2.85 g), although not significantly different from the 12-hour LED exposure treatment. The results of this study are consistent with the results of Samuolienė et al. [10], who conducted research on microgreen kohlrabi and mizuna cultivation. LED light may have little effect on the dry weight of Vigna radiata microgreens because their short development cycle limits the time for significant biomass formation, and the plants may reach a saturation point in photosynthesis where additional light does not increase dry weight [2]. Additionally, the light's energy could be directed toward other areas of growth, such as improving quality rather than increasing dry weight

Table 2. Mean of dry weight of mung bean microgreen due to LED exposure duration and dark phase treatment.

LED treatment	Dry Weight (g)		
	Total DW	Shoot DW	Root DW
Duration (L)			
0 Hour	2,31 a	2,11 ab	0,20
6 Hours	2,17 a	1,96 a	0,21
12 Hours	2,83 b	2,48 bc	0,35
18 Hours	3,14 b	2,85 c	0,29
BNT 5%	0,54	0,51	tn
Dark Period Treatment(B)			
Without Dark Period	2,58	2,28	0,30 b
Using dark period	2,64	2,42	0,22 a
LSD 5%	ns	Ns	0,06

Notes: Numbers accompanied by the same letter in the column show no significant difference according to the BNT test at the 5% level; tn = not significant.

The variance test results showed no significant interaction between the LED exposure duration treatment and the dark phase treatment to total soluble solids (TPT), total vitamin C, and chlorophyll. However, there was a significant interaction between the two treatments on microgreen texture. Regardless, the treatment duration of LED exposure significantly impacted total soluble solids 4 and 6 days after planting. The dark phase treatment only showed a significant effect when the plants were 4% of the total soluble solids variable. The results of the 5% LSD test in Table 9 show that the 18-hour exposure treatment produced microgreens with the highest total soluble solid values (4.40ºBrix and 4.13ºBrix), not significantly different from the 12-hour radiation treatment. However, it was significantly different from the other treatments. The result showed that extended exposure to LED light for 12-18 hours increases the accumulation of total soluble solids in Vigna radiata microgreens due to the extension of the photosynthetic process, which increases the synthesis of sugars and other soluble chemicals. This finding is supported by empirical research, which shows that extended photoperiods increase carbohydrate synthesis in plants, resulting in a higher concentration of total soluble solids [11] [12]. Regardless, the microgreen that did not receive dark phase treatment produced the best total soluble solids value (3.83°Brix) at four days after planting, which was significantly different from microgreen plants that received dark phase treatment. The two treatments separately had no significant effect on the vitamin C variable. However, the dark phase treatment significantly affected the total chlorophyll variable at four days after planting. This phenomenon may be caused by a physiological reaction of the Vigna radiate microgreen, in which chlorophyll synthesis is reduced in the early stages. However, when the microgreens are exposed to light, the plants quickly increase chlorophyll production to optimize photosynthesis by day four and return to normal after a period of time.

Table 3. Mean of total dissolved solids of mung bean microgreen due to the treatment of led exposure length and dark phase treatment

LED treatment	Total Soluble Solid (ºBrix)	
	4 DAP	6 DAP
Duration (L)		
0 Hour	2,75 a	2,72 a
6 Hours	2,80 a	3,03 a
12 Hours	4,17 b	3,50 b
18 Hours	4,40 b	4,13 b
BNT 5%	0,67	0,65
Dark Period Treatment(B)		
Without Dark Period	3,83 b	3,47
Using dark period	3,22 a	3,23
LSD 5%	0,42	tn

Notes: Numbers accompanied by the same letter in the column show no significant difference according to the BNT test at the 5% level; tn = not significant.

Table 4. Mean of chlorophyll of mung bean microgreen as treated with a long time of LED exposure and dark phase treatment

LED treatment	Chlorophyll (µg/ml)	
	4 DAP	6 DAP
Duration (L)		
0 Hour	19,41	34,67
6 Hours	18,70	36,32
12 Hours	24,00	44,82
18 Hours	27,78	46,04
BNT 5%	ns	ns
Dark Period Treatment(B)		
Without Dark Period	19,39 a	42,39
Using dark period	25,55 b	38,54
LSD 5%	4,90	ns

Notes: Numbers accompanied by the same letter in the column show no significant difference according to the BNT test at the 5% level; tn = not significant.

The analysis of variance of plant texture showed a significant interaction between the combination of LED exposure and dark phase treatment. The results of the 5% LSD test in Table 11 show that the treatment combination of 18 hours of LED exposure with blackout treatment produces the highest average value of microgreen texture at 6.10 mm/s, not significantly different from other treatment combinations. However, it is significantly different from the 18-hour treatment combination without blackout treatment (2.03 mm/s).

Table 5. Mean of the texture of mung bean microgreen due to the duration of LED exposure and dark phase treatment

Treatments		Texture (mm/s)
Duration of LED Exposure	Dark Phase	
0 Hour	Without Dark Phase	5,33 b
	Using Dark phase	4,37 ab
6 Hours	Without Dark Phase	4,17 ab
	Using Dark phase	4,87 b
12 Hours	Without Dark Phase	4,40 ab
	Using Dark phase	3,73 ab
18 Hours	Without Dark Phase	2,03 a
	Using Dark phase	6,10 b
LSD 5%		2,42

Notes: Numbers accompanied by the same letter in the column show no significant difference according to the BNT test at the 5% level; tn = not significant.

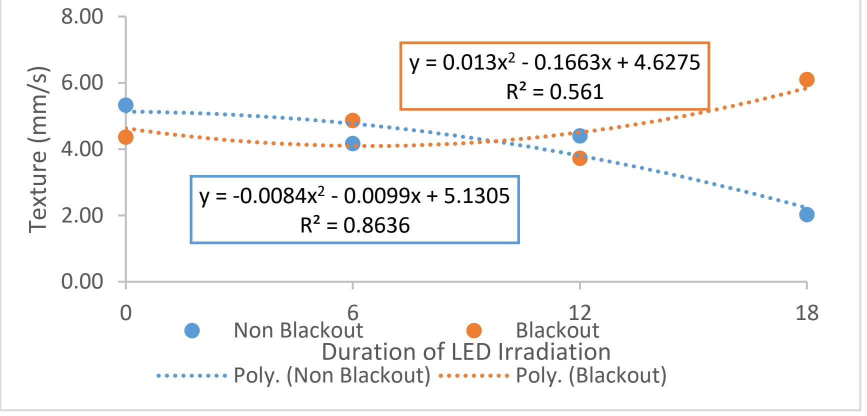

Figure 1. Regression Analysis of Tevture variable Due duration of LED Exposure and Dark Phase Treatment

The regression analysis results on the treatment without blackout (Figure 1) revealed a quadratic pattern, with the equation $y = -0.0084x^2 - 0.0099x + 5.1305$ and the coefficient of determination (R^2) = 0.8636. It indicated that the treatment involving LED exposure durations ranging from 0 to 18 hours without blackout resulted in a decreasing trend. Consequently, the texture of mung bean microgreens reduced vegetable hardness with each additional length of exposure. Conversely, the regression analysis on the blackout treatment yielded a quadratic pattern with the equation $y = 0.013x^2 - 0.1663x + 4.6275$ and the coefficient of determination (R^2) = 0.561. It was discovered that the hardness of mung bean microgreen texture rose initially until it reached an ideal point, after which it decreased. Patida et al. [13] stated that mung bean seeds germinated in darkness showed increased hardness initially, likely due to the accumulation of structural compounds and proteins during the early stages of germination. The hardness peaked as the microgreens developed, reflecting optimal growth conditions and nutrient accumulation [14].

4 Conclusion

The study results show that the combined treatment of 18 hours of LED exposure with a dark period resulted in the highest plant texture value (6.10 mm/s). Variations in light duration significantly impacted total fresh weight, total dry weight, shoot dry weight, and total soluble solids at 4 and 6 days after planting. Specifically, 12-hour LED irradiation resulted in the highest total fresh weight (85.15 g). In comparison, 18-hour exposure resulted in the highest total dry weight (6.28 g), shoot dry weight (5.70 g), and total soluble solids for both led 4 and 6 days after planting (8.80 °Brix or 8.27 °Brix). Meanwhile, the dark phase treatment resulted in the highest chlorophyll content (68.23 μg/cm²) 4 days after planting. Mung bean microgreens grown without an initial dark period showed the highest root dry weight (0.89 g) and total soluble solids (11.50 °Brix) 4 days after planting.

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