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Physiological and Biochemical Responses of *Bisbul* (*Diospyros discolor* Willd.) Seedlings to Varying Artificial Light at Night (ALAN)

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ABSTRACT

Artificial light at night (ALAN) can significantly affect plant physiology, as many physiological processes are light-dependent. However, studies investigating the specific effects of ALAN on plants remain limited. This study aimed to assess the impact of ALAN on the growth and metabolite composition of *bisbul* (*Diospyros discolor* Willd.). The experiment was conducted in Nursery 2 of the Bogor Botanical Gardens over 12 months. A split-plot factorial design was employed with three replications, each consisting of three one-year-old seedlings. The main plots were assigned to light color treatments (control, red, green, and blue), subplots to light intensity levels (control, high, and low), and sub-subplots to illumination durations (0, 1, 6, or 12 hours) applied for 0, 2, or 7 nights/week. ALAN treatments were administered continuously for one year. The results indicated that blue light significantly increased leaf senescence, particularly under BH-6(2), BH-12(2), and BH-12(7) treatments. Conversely, high-intensity red light reduced plant height, shoot dry mass, photosynthetic rate, and chlorophyll content. Metabolite profiling revealed decreased levels of secondary metabolites such as caffeic acid and catechin, while compounds such as nicotinamide, L-proline, linolenic acid, and coumarin increased. These findings suggest that prolonged exposure (6-12 hours) to high-intensity red or blue light can disrupt circadian rhythms and impair physiological functions.

1. Introduction

Light is one of the abiotic factors that is very important in several physiological processes of plants. Plants need light as an energy source to carry out the process of photosynthesis. In addition, light also affects several physiological processes critical in growth and development, such as germination, flowering, senescence, and so on (Van Gelderen *et al.* 2018; Wu *et al.* 2024). However, the intensity and duration of ALAN exposure can impact plants differently. This difference is caused by differences in wavelength, color spectrum, and duration of exposure, which affect the plant's ability to capture energy from the light and impact changes in plant metabolism (Agnestika *et al.* 2017).

Artificial light at night (ALAN) refers to artificial illumination of various colors that, upon exposure to plants, disrupts physiological processes and leads to deviations from normal conditions. ALAN is generally found in areas with high mobility, such as highways, public roads, buildings, billboards, etc. One of the impacts of ALAN is changes in the biological clock's rhythm (*Circadian rhythm*). Several studies have shown

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that ALAN hurts plant growth and productivity. Plants exposed to ALAN show changes in photosynthesis rates, increased respiration rates, and the emergence of oxidative stress.

Research on the impact of ALAN on plants in subtropical areas shows that plants experience accelerated leaf fall. The plants also show accelerated leaf initiation compared to non-ALAN conditions (Meng *et al.* 2022). Exposure to high-intensity ALAN has been shown to reduce photosynthetic efficiency, increase oxidative stress in plants, and disrupt the accumulation of secondary metabolites, with the opposite results obtained under optimal conditions being reduced (Friulla and Varone 2025). In addition, exposure to ALAN, particularly red and blue light, has been shown to increase the accumulation of secondary metabolites in *Tagetes tenuifolia*, such as flavonoids, carotenoids, and polyphenols (Appolloni *et al.* 2022), which are used as a defense mechanism against biotic and abiotic stress (Zhang *et al.* 2021).

In 2021, ALAN was used for night tourism at the Bogor Botanical Gardens (KRB). Research on the impact of ALAN on plants in tropical areas is still rare. Therefore, it is necessary to research the effect of ALAN on plants, especially for plant conservation areas, such as KRB, which is planned to be expanded with night activities involving the use of ALAN. In addition to functioning as a tourist attraction, the KRB serves as one of the ex-situ plant conservation areas, protecting endangered species through recorded collections and maintenance (Irawanto 2023). Therefore, maintaining the ecosystem is crucial to ensure the sustainability of the plant collection. KRB has various plant collections, including *Bisbul* (*Diospyros discolor* Willd.).

Bisbul is a species endemic to tropical regions, originating from the Philippines and belonging to the Ebenaceae family. *Bisbul*, often referred to as butter fruit, is frequently found in the Bogor area as a local fruit. *Bisbul* is a tree species usually planted on roadsides or in parking areas. This plant generally has a height of up to 15 m, with a straight trunk that is reddish-brown to black. The oval-shaped leaves are arranged alternately, and the underside is finely hairy. The fruit is oval-shaped, ripe fruit is reddish brown, the flesh is soft like butter, and it has a sweet taste (Gunawan and Partomihardjo 2019; Ernawati and Purwaningsih 2022). The fruit has a smooth, hairy surface, can be consumed directly, and has high economic value. *Bisbul* has many economic potentials, such as the wood's commercial value, making *Bisbul* one of the superior horticultural commodities that could be further developed (Deswina *et al.* 2019). The fruit and plant tissues of *Bisbul* are known to contain crucial secondary metabolites such as alkaloids, tannins, flavonoids, and phenolics (Noviardi *et al.* 2019), which showed significant antioxidant, anti-inflammatory, analgesic, anti-diarrhoeal, and antimicrobial activities (Haque *et al.* 2020).

Bisbul has not only economic importance but also significant ecological value due to its adaptation to humid tropical environments and low light intensity. This makes it highly sensitive to environmental light disturbances, including artificial light at night (ALAN). The selection of *Bisbul* as the subject of this study is based on the limited information regarding its physiological and metabolite responses to environmental stressors such as ALAN, as well as its relevance as a representative of understudied tropical species in this context. *Bisbul* growing in the Bogor Botanical Gardens (KRB) is also potentially affected by night lighting programs implemented for tourism. Until now, no comprehensive study has evaluated the impact of ALAN on *Bisbul*. Based on this background, this study aims to analyze the effect of variations in color, intensity, and duration of ALAN illumination on the growth, physiological, and secondary metabolite aspects of *Bisbul*, as well as to evaluate the possibility of disruption of physiological and metabolic rhythms due to ALAN. In addition, this study aims to provide scientific information on the tolerance and response of rare tropical plants, such as *Bisbul*, to light pollution, which can serve as a basis for recommendations on lighting system management in conservation areas, particularly in the Bogor Botanical Gardens.

2. Materials and Methods

2.1. Research Location

The study was conducted at the Bogor Botanical Gardens, West Java, Indonesia. Physiology and metabolite profile analyses were performed at IPB University, Indonesia.

2.2. Research Design

The research design used a split-plot design with a randomized block design factorial with three replications. Each research unit consists of three *Bisbul* seedlings. Treatment consists of color light, light illumination, and light intensity. The main plot is colored light (control, red, green, blue). The subplot was light intensity (0, high, and low). Sub-sub plot was illumination duration (0, 1,

6, or 12 hours for 0, 2, and 7 nights). The type and code of treatments are listed in Table 1. Average intensity light is as follows: 0.60 Wm^{-2} (red-high), 0.13 Wm^{-2} (red-low), 0.49 Wm^{-2} (green-high), 0.13 Wm^{-2} (green-low), 0.30 Wm^{-2} (blue-high), and 0.06 Wm^{-2} (blue-low). The intensity of light is measured at night and during the day using a lux meter that has been calibrated.

The *Bisbul* seedlings used are one year old. Seedlings obtained from KRB were placed in 19 plots, with a distance of 50 cm. Each plot measuring 120 cm × 120 cm × 230 cm is made from a light steel frame and consists of two light intensity levels. In a way, every plot randomly accommodates three plants on every level with varying light intensity levels. Plants were maintained under a natural day–night cycle without additional light treatment. The lighting system was controlled automatically using a digital timer programmed according to the treatment schedule. Lights were turned on at 7 p.m. for all treatments, except for the 12-hour duration treatment, where the lights were switched on at 6 p.m. Control plants were kept under natural dark conditions at night. The placement of experimental units was arranged to ensure that illumination occurred according to the assigned treatment durations. Illumination consistency was monitored continuously, both day and night, using CCTV cameras installed facing each plant rack. This system ensured the accuracy and stability of the treatment lighting for each plant.

Table 1. The treatment types and codes in the research

Light color	Light intensities	Light durations	Treatment codes
Control		0	C
Red	High (7.83 lux)/ Low (16.65 lux)	1 h/2 nt/w	RH-1(2)/RL-1(2)
		1 h/7 nt/w	RH-1(7)/RL-1(7)
		6 h/2 nt/w	RH-6(2)/RL-6(2)
		6 h/7 nt/w	RH-6(7)/RL-6(7)
		12 h/2 nt/w	RH-12(2)/RL-12(2)
		12 h/7 nt/w	RH-12(7)/RL-12(7)
Green	High (62.07 lux)/ Low (15.93 lux	1 h/2 nt/w	GH-1(2)/GL-1(2)
		1 h/7 nt/w	GH-1(7)/GL-1(7)
		6 h/2 nt/w	GH-6(2)/GL-6(2)
		6 h/7 nt/w	GH-6(7)/GL-6(7)
		12 h/2 nt/w	GH-12(2)/GL-2(2)
		12 h/7 nt/w	GH-12(7)/GL-12(7)
Blue	High (3.42 lux)/ Low (7.52 lux	1 h/2 nt/w	BH-1(2)/BL-1(2)
		1 h/7 nt/w	BH-1(7)/BL-1(7)
		6 h/2 nt/w	BH-6(2)/BL-6(2)
		6 h/7 nt/w	BH-6(7)/BL-6(7)
		12 h/2 nt/w	BH-12(2)/BL-12(2)
		12 h/7 nt/w	BH-12(7)/BL-12(7)

h: hour, nt: night, w: week. RH: Red-High, RL: Red-Low, GH: Green-High, GL: Green-Low, BH: Blue-High, BL: Blue-Low. 1(2): 1 hour/2nights, 1(7): 1 hour/7nights, 6(2): 6 hours/2nights, 6(7): 6 hours/7nights, 1(2): 12 hours/2nights, 12(7): 12hours/7nights

2.3. Growth Observations

Growth of *Bisbul* was carried out by measuring the increase in plant height and the number of senescing leaves every month. The shoot dry weight was observed 12 months after treatment (MAT).

2.4. Chlorophyll Content

The total chlorophyll content in *Bisbul* was measured at 12 MAT of ALAN using the method of Lichtenthaler *et al.* (1981) at wavelengths (λ) of 663 and 647 nm. The 0.1 g of mature leaf was extracted with 80% p.a. acetone. The samples were centrifuged at 2,500 rpm for 10 minutes at 4°C (Heraeus Labofuge 400R, Germany). The absorbance of the supernatant was measured using a Spectrophotometer Thermospectronic Genesys 20 (Thermo, USA). The chlorophyll content was calculated using the following formula:

$$\text{Chlorophyll a} = 12.25\ A_{663} - 2.79\ A_{647}$$
$$\text{Chlorophyll b} = 21.50\ A_{647} - 5.10\ A_{663}$$
$$\text{Total chlorophyll} = 7.15\ A_{663} + 18.71\ A_{647}$$

2.5. Photosynthesis Rate

Photosynthesis rate was measured on mature leaves every 3 months (3, 6, 9, and 12 MAT) with LI-COR 6400 Portable Photosynthesis System (LI-COR Inc., Lincoln, Nebraska, USA) at PAR (Photosynthetically Active Radiation) 1,000 µmol m^{-2} s^{-1} with temperature at 30°C, humidity 60%, and CO_2 400 ppm.

2.6. Metabolite Profile Extraction and Analysis

Leaf samples were taken after 12 MAT. Leaves from each experimental unit were taken, washed with tap water, and dried at 40-50°C for 10 days. The 5 g of leaf powder was extracted with 50 mL of 70% ethanol p.a and incubated using a shaker for 72 hours at 120 rpm at room temperature. The extract was filtered to obtain the filtrate using the Whatman No. 4 filter paper. The filtrate was evaporated using a rotary evaporator at 40°C until a paste was obtained. The macerated sample was transferred to a new tube and analyzed by LC-MS/MS. LC-MS/MS analysis was performed according to the Advanced Laboratory IPB protocol. A total of 2 µL of sample solution with a concentration of 10 mg mL^{-1} was injected into the LC-MS/MS device (UHPLC Vanquish Tandem Q Exactive Plus Orbitrap HRMS Thermoscientific) using an Accucore C18 column, 100 × 2.1 mm, 1.5 µm (Thermoscientific). The reaction was carried out at a column temperature of 30°C and a flow rate of 0.2 mL min^{-1}. The mobile phase consists of phase

A (H_2O in 0.1% formic acid) and phase B (acetonitrile + 0.1% formic acid), which are arranged in several gradients, namely 0-1 minute (5%B), 1-25 minutes (5-95% B), 25-28 minutes (95% B), and 28-35 minutes (5% B).

2.7. Data Analysis

Data analysis was performed using Analysis of Variance ANOVA at a 95% confidence level using RStudio 4.2.1 software. If there was a significant difference, further testing was carried out using the Duncan Multiple Range Test (DMRT) with a level of $\alpha = 5\%$. Metabolite profile data were analyzed and identified using MZmine software version 3.9.0. Identification results were visualized using the MetaboAnalyst 6.0 page. Compound identification was conducted using the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

3. Results

3.1. Growth Changes in *Bisbul* Affected by ALAN

The light treatment with various colors and intensities given as a form of ALAN treatment caused different growth responses in *Bisbul* plants. The increase in the height of the *Bisbul* was influenced by the interaction between light color and light intensity ($p<0.05$) (Table 2). Plant height increased in the high-intensity red-light treatment but was lower than that of the control. In contrast, the blue-light treatment at high and low intensity was not different from the control. The green light, both at high and low intensity, also did not provide significant differences ($p<0.05$). In addition, the *Bisbul* shoot dry weight changed in response to light intensity and color, each having a separate effect ($p<0.05$). The red, green, and blue light treatments were lower than the control. The shoot dry weight of the high and low light intensity treatments was also lower than that of the control. Both high and low-intensity treatments also showed lower shoot dry weight than that of the control (Table 3).

Artificial light at night (ALAN) significantly affected the number of senescence leaves in *Bisbul* during 12 months of treatment, which was influenced by the interaction between light color, intensity, and light duration ($p<0.05$) (Table 4). In general, most combinations of light treatments led to an increase in the number of senescent leaves compared to the control. The highest senescence was observed under high-

Table 2. Height Increase in the bisbul plant height after 12 months of ALAN treatments with varying light colors and intensities

Light color	Light intensity	Increase in plant height (cm)
Control		55.67^{a}
Red	High	42.56^{b}
	Low	55.11^{a}
Green	High	55.67^{a}
	Low	52.89^{a}
Blue	High	50.50^{ab}
	Low	50.22^{ab}

Numbers in the same column followed by different letters indicate significant differences in the DMRT test (α: 5%)

Table 3. Measure the dry weight of the bisbul after 12 months of ALAN treatment under varying light conditions

Factor	Shoot dry weight (g)
Light Color	
Control	5.49^{a}
Red	5.06^{c}
Green	5.16^{b}
Blue	5.15^{b}
Light Intensity	
Control	5.49^{a}
High	5.09^{b}
Low	5.15^{b}

Numbers in the same column followed by different letters indicate significant differences in the DMRT test (α: 5%). Data were transformed using log 10 (x)

Table 4. Number of senescence leaves in bisbul after 12 months of ALAN treatment with varying light colors, durations, and light intensities

Light color	Light durations	Number of senescence leaves	
		Light intensities	
		High	Low
Control		13.33^{j-n}	13.33^{j-n}
Red	1 h/2 nt/w	12.00^{mn}	11,33^{n}
	1 h/7 nt/w	24.33^{ab}	13.33^{j-n}
	6 h/2 nt/w	19.67^{b-g}	17.00^{d-l}
	6 h/7 nt/w	15.67^{g-n}	19.00^{c-i}
	12 h/2 nt/w	18.00^{d-j}	13.33^{j-n}
	12 h/7 nt/w	20.33^{b-g}	18.33^{d-l}
Green	1 h/2 nt/w	13.00^{k-n}	11.67n
	1 h/7 nt/w	16.67^{e-m}	12.00^{mn}
	6 h/2 nt/w	16.00^{f-n}	17.00^{d-l}
	6 h/7 nt/w	20.67^{b-f}	14.67^{h-n}
	12 h/2 nt/w	16.67^{e-m}	21.33^{b-e}
	12 h/7 nt/w	21.33^{b-e}	17.67^{d-k}
Blue	1 h/2 nt/w	12.67^{l-n}	14.33^{i-n}
	1 h/7 nt/w	17.00^{d-l}	12.00^{mn}
	6 h/2 nt/w	21.67^{a-d}	20.00^{b-g}
	6 h/7 nt/w	19.67^{b-g}	21.00^{b-e}
	12 h/2 nt/w	26.00^{a}	19.33^{c-h}
	12 h/7 nt/w	23.33^{a-c}	19.67^{b-g}

h: hour, nt: night, w: week. Numbers in the same column followed by different letters indicate significance in the DMRT test (α:5%)

intensity blue-light treatments with long durations (6 and 12 hours), particularly in BH-6(2), BH-12(2), and BH-12(7). Additionally, several other treatments also showed a notable increase in leaf senescence, including RH-1(7), RL-6(7), RH-12(7), GH-6(7), GL-12(2), and GH-12(7).

3.2. Physiological Response of *Bisbul* to ALAN Illumination

The rate of photosynthesis in *Bisbul* was significantly influenced by the interaction between light color, light intensity, and duration of illumination ($p<0.05$) (Table 5). In red-light treatments, the photosynthesis rate varied depending on the combination of duration and intensity. The high-intensity red-light treatment with a duration of 12 hours for 7 nights/week (R-12(7)) resulted in a photosynthesis rate of 30.83 µmol CO_2 $m^{-2}s^{-1}$, which was not significantly different from the control (30.24 µmol CO_2 $m^{-2}s^{-1}$). Similarly, total chlorophyll content in *Bisbul* was significantly affected by the interaction of light color, intensity, and illumination duration ($p<0.05$) (Table 5). Green-light treatments generally resulted in higher chlorophyll content than red or blue-light, particularly under the 1-hour/7-night high-intensity condition (26.78 mg g^{-1} FW). In contrast, red-light treatments such as RH-6(7) and RH-12(7) significantly reduced chlorophyll content. Some blue-light treatments, specifically the 1-hour/7-night high-intensity condition, produced even higher chlorophyll content (27.54 mg g^{-1} FW) than the control (22.26 mg g^{-1} FW).

3.3. Metabolite Response of *Bisbul* to ALAN Illumination

Metabolite profile analysis was conducted after 12 months of treatment. The analysis focused on three representative treatments that showed the most severe and the mildest effects on growth for each light color (red, green, and blue), based on their intensity and duration: H-12(7), H-6(7), and L-1(7). *Bisbul* exposed to high-intensity ALAN exhibited significant changes in metabolite concentrations. Treatments with 6- and 12-hours of exposure/night, 7 nights/week, had adverse effects, whereas the 1-hour/7-night treatment and the control showed no significant impact. Several metabolites showed altered concentrations in response to ALAN treatments across all light colors. The concentrations of caffeic acid and catechin decreased in all ALAN-treated plants (red, green, and blue) compared to the control. In contrast, metabolites such as nicotinamide, L-proline, linolenic acid, and coumarin increased in concentration relative to the control (Figure 1).

Table 5. Photosynthesis rate and total chlorophyll content of bisbul after 12 months of ALAN treatment with varying light colors, durations, and light intensities

Light color	Light durations	Photosynthesis rate (µmol CO_2 m^{-2} s^{-1})		Total chlorophyll (mg. g^{-1} fw)	
		Light intensities			
Control		High	Low	High	Low
		30.24^{a-c}	30.24^{a-c}	22.26^{c-h}	22.26^{c-h}
Red	1 h/2 nt/w	29.29^{a-d}	28.54^{a-d}	25.68^{a-b}	23.77^{a-c}
	1 h/7 nt/w	30.24^{a-c}	29.28^{a-d}	18.38^{j-n}	19.45^{h-l}
	6 h/2 nt/w	29.21^{a-d}	27.15^{c-d}	15.69^{n-p}	22.65^{c-g}
	6 h/7 nt/w	27.02^{c-d}	28.64^{a-d}	10.02^{q}	16.32^{m-p}
	12 h/2 nt/w	30.05^{a-c}	28.72^{a-d}	23.39^{b-f}	17.13^{l-p}
	12 h/7 nt/w	30.83^{a-b}	26.56^{d}	14.27^{p}	19.06^{i-m}
Green	1 h/2 nt/w	29.37^{a-d}	30.63^{a-b}	22.73^{b-f}	20.56^{f-j}
	1 h/7 nt/w	30.83^{a-b}	28.74^{a-d}	26.78^{a}	18.30^{j-n}
	6 h/2 nt/w	28.51^{a-d}	30.82^{a-b}	17.43^{k-o}	21.80^{d-i}
	6 h/7 nt/w	30.08^{a-c}	30.08^{a-c}	15.16^{o-p}	23.29^{b-f}
	12 h/2 nt/w	28.13^{a-d}	30.84^{a-b}	23.39^{b-f}	18.95^{i-m}
	12 h/7 nt/w	31.15^{a}	30.66^{a-b}	21.57^{d-i}	20.36^{f-k}
Blue	1 h/2 nt/w	29.91^{a-c}	28.13^{a-d}	24.99^{a-b}	19.26^{h-m}
	1 h/7 nt/w	28.62^{a-d}	29.24^{a-d}	27.54^{a}	22.65^{c-g}
	6 h/2 nt/w	27.57^{b-d}	30.15^{a-c}	23.73^{b-e}	18.25^{j-n}
	6 h/7 nt/w	28.60^{a-d}	30.82^{a-b}	15.21^{o-p}	17.87^{j-o}
	12 h/2 nt/w	27.72^{b-d}	30.87^{a-b}	17.25^{l-o}	19.66^{h-m}
	12 h/7 nt/w	27.82^{a-d}	29.24^{a-d}	20.70^{e-j}	21.40^{d-i}

h: hour, nt: night, w: week. Numbers in the same column followed by different letters indicate significance in the DMRT test (α: 5%)

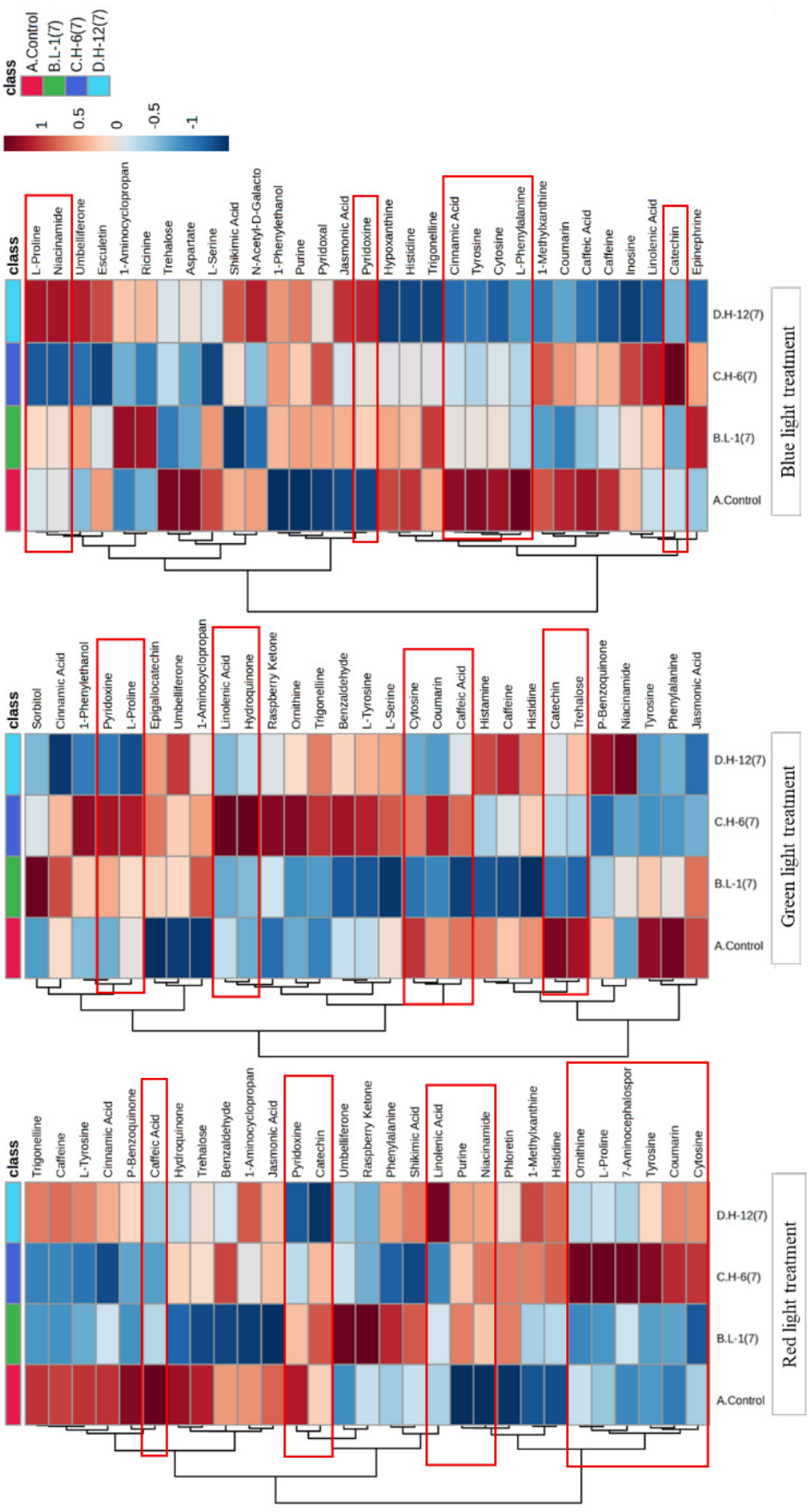

Figure 1. Heatmap of metabolite profile affected by ALAN after 12 months of treatment. L-1(7): low intensity 1 hour/7 nights; H-6(7): high intensity 6 hours/7 nights; H-12(7): high intensity 12 hours/7 nights

4. Discussion

Plants naturally require a dark period at night to facilitate cellular repair processes that address metabolic damage accumulated during the day. In *Bisbul*, exposure to high-intensity red and blue light disrupted this balance, resulting in a significantly lower increase in plant height compared to the control. However, the high-intensity blue light treatment alone did not show a statistically significant difference from the control. This response may be attributed to the inhibition of chlorophyll biosynthesis and photosynthetic enzyme activity, which affects pigment composition and ultimately hinders plant growth (Speißer *et al.* 2021; Dianursanti *et al.* 2025). A similar effect has been observed in *Coriandrum sativum* exposed to high-intensity red and blue light. These findings suggest that the loss of nighttime darkness due to ALAN exposure disrupts the plant's circadian rhythm, which is crucial for regulating cell repair and energy recovery. *Circadian rhythm* disruption can also interfere with the synthesis of hormones essential for plant growth

Artificial light at night disrupts the circadian rhythm, thereby altering the expression of genes that regulate photosynthesis, such as CAB (chlorophyll a/b-binding protein) and RBCS (ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit), thus causing the formation of free radicals (ROS), which can damage cellular structures and DNA, and trigger prolonged stress (Bennie *et al.* 2016; Venkat and Muneer 2022). Stress induced by ALAN can disrupt the biosynthesis of key hormones such as auxin and cytokinin, which are essential for cell differentiation. These findings are consistent with those of Wei *et al.* (2023), who reported that ALAN exposure in tropical plants such as *Euonymus japonicus* and *Rosa hybrida* led to decreased photosynthetic efficiency, increased reactive oxygen species (ROS) production, and metabolic disturbances that inhibit plant growth and development. Nighttime light stress can increase ROS levels (Bennie *et al.* 2018). If the plant's antioxidant system does not effectively neutralize these, they may lead to chlorophyll degradation and reduction in the synthesis of secondary metabolites, including phenols and flavonoids. Therefore, ALAN not only directly affects photosynthesis but also interferes with molecular and hormonal regulation, both of which are critical for plant growth and resilience.

Chlorophyll biosynthesis is a complex process influenced by environmental conditions, including lighting (Yong *et al.* 2024). ALAN treatment of red, green, and blue light with durations of 6 and 12 hours, 7 nights a week, caused a decrease in total chlorophyll content. This decrease occurred due to disruption of the circadian rhythm, which regulates 5-aminolevulinic acid (ALA) production and enzyme activity for chlorophyll synthesis. As a result, the chlorophyll levels of plants exposed to ALAN were lower than those of the control. However, ALAN treatment with a shorter duration allowed chlorophyll levels to remain stable. This decrease in chlorophyll has a direct impact on the rate of photosynthesis. *Bisbul* was exposed to red light for 12 hours/7 nights/week at low intensity and had a lower photosynthesis rate after 12 MAT than the control. This occurs because the illumination duration is too long, which causes damage to the chloroplast structure, resulting in decreased photosynthetic efficiency (Gloria *et al.* 2018), thus potentially reducing the energy supply needed for growth. Similar conditions are seen in *Populus alba*, which is given lighting at night, which can reduce CO_2 assimilation in the morning (Lo Piccolo *et al.* 2024). Assimilation influences carbon allocation, which in turn affects dry weight, leaf senescence, and the production of defense compounds in *Bisbul* under stress from ALAN exposure (Cao *et al.* 2024).

A significant decrease in shoot dry weight was observed in the *Bisbul* exposed to ALAN red, green, and blue light, which was lower than in the control. This occurs because the photosynthates generated through photosynthesis are also used for functions other than growth, such as stress response and defense. However, photosynthate is also used to create new secondary metabolite compounds or increase the compound content for self-defense from ALAN, such as nicotinamide, l-proline, linolenic acid, and coumarin. Metabolite compounds such as caffeic acid and catechin experienced a decrease in concentration that occurred in the treatment of three light colors (red, green, and blue). Caffeic acid and catechin are known as phenolic compounds, critical secondary metabolites, such as ALAN, that protect plants from environmental stress (Bernatoniene and Kopustinskiene 2018; Lubis *et al.* 2025). The decrease in these compounds indicates that the *Bisbul* is trying to optimize defense due to unnatural lighting, which reflects the complex interaction between growth and metabolic regulation in dealing with abiotic stress (Ramzan *et al.* 2024). This shows that providing light during the night causes an increase in the production of reactive oxygen species (ROS), which triggers oxidative stress and inhibits

the activity of enzymes needed for metabolism that controls growth (Wang *et al.* 2024). This disturbance decreases photosynthate accumulation in the shoot, so the dark period at night is essential for the physiological recovery process of plants and metabolic regulation (Lo Piccolo *et al.* 2024). This has implications for the accelerated senescence process and the adjustment of metabolites to increase the resistance of *Bisbul* to suboptimal environmental conditions.

Another impact of ALAN treatment on *Bisbul* is the occurrence of premature leaf aging (premature senescence). *Bisbul* exposed to blue light showed the highest leaf senescence, especially in the BH-12(2) treatment, when compared to red, green, and control light treatments. However, blue light is often associated with chlorophyll degradation. The results showed that the treatment BH-1(7) had the highest chlorophyll content. These findings indicate that the increase in senescent leaves is not confined to specific conditions but occurs across various combinations of light color, intensity, and duration. The interaction between light color, intensity, and duration greatly influences the plant's response to senescence, which is likely also triggered by other stresses, such as increased ROS due to night lighting (Kwak *et al.* 2018). In addition, ALAN triggers the formation of reactive oxygen species (ROS), especially superoxide anion (O_2-). The accumulation of ROS causes cellular damage, including membranes, proteins, and DNA, thus affecting the physiological function of leaves (Sakuraba 2021). This condition indicates that ALAN disrupts basic physiological processes and can cause a general metabolic imbalance in tropical plants.

Bisbul exhibits physiological adaptations that influence the increase or decrease in metabolite concentrations under different treatments. The concentrations of cytosine and tyrosine metabolites decreased in plants exposed to GH-12(7) and BH-12(7) compared to the control. Tyrosine is involved in protein synthesis, while cytosine plays a role in cell division and differentiation, influencing plant hormones such as abscisic acid (ABA) and ethylene (Liu *et al.* 2023).

The decrease in the concentration of these metabolites is related to premature senescence in leaves. This can potentially accelerate senescence through the induction of programmed cell death. ABA and ethylene trigger senescence in leaves, which eventually causes leaves to fall due to ALAN exposure. The resulting oxidative stress can disrupt the balance of metabolism, including vitamin B6 metabolism, also known as pyridoxine metabolites. In conditions of oxidative stress due to ALAN, pyridoxine metabolites in the red and green light decrease. Pyridoxine is used to neutralize oxidative damage (Czegeny *et al.* 2019). This damage will undoubtedly affect the rate of photosynthesis by regulating the expression of genes involved in growth. On the other hand, umbelliferone is formed in green and blue light with a duration of 6 and 12 hours/7 nights, functioning as an antioxidant compound that can reduce damage caused by ROS and protect plant cells from oxidative stress (Kornicka *et al.* 2023), and the concentration of ornithine metabolites also increases. This compound can contribute to the efficiency of energy use in plants exposed to artificial light (Sivakumar *et al.* 2022).

ALAN's exposure to light treatments (red, green, and blue) also affects the formation of nicotinamide metabolite compounds. It increases the concentration of metabolites in the three lights (red, green, and blue), namely L-proline, linolenic acid, and coumarin. Coumarin in plants regulates growth and metabolism as a defense mechanism (Robe *et al.* 2021). In addition, increasing nicotinamide concentrations helps protect plant tissues from ROS damage and has a DNA hypomethylation effect, affecting defense gene expression (Berglund *et al.* 2017). This suggests that coumarin and nicotinamide play essential roles in maintaining cellular health. However, at the same time, they may inhibit *Bisbul* growth, leading to a smaller increase in plant height compared to the control. Oxidative stress triggers an increase in L-proline and linolenic acid levels. L-proline increases stress tolerance by protecting cells from oxidative damage and maintaining osmotic balance (Hosseinifard *et al.* 2022; Liu *et al.* 2023), while linolenic acid supports photosynthesis and affects cell membrane fluidity for cell function (Takic *et al.* 2024). Stress conditions generally stimulate the production of secondary metabolites in plants. Therefore, the changes observed in *Bisbul* suggest a negative impact.

Exposure to ALAN negatively affects *Bisbul*, particularly under red and blue light treatments with long durations and high intensities, such as 6 or 12 hours/night for 7 consecutive nights. These treatments showed decreased photosynthetic efficiency, increased senescence, and accumulation of oxidative stress, potentially leading to tissue death and defoliation. In contrast, green light with short duration and low intensity, such as 1 hour/2 nights/week, tends to show milder physiological impacts on plants. The sustained

decline in photosynthetic efficiency, caused by cellular damage and prolonged oxidative stress, poses a serious threat to plant survival, particularly in conservation areas. Therefore, regulating the duration and intensity of artificial light at night is crucial. Selecting light colors with minimal adverse effects and maintaining a sufficient dark period are essential to support cellular recovery and ensure optimal plant growth.

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