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The Effect of Different Proportions of Blue Light on Some Developmental and Physiological Characteristics of Nasturtium (*Tropaeolum majus* L.)

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ABSTRACT

The spectral quality of light is critical for modulating plant morphology and physiology in the absence of sunlight. This study investigated the influence of varying red (R) to blue (B) light ratios on the growth, morphology, and physiological performance, such as photosynthetic efficiency and chlorophyll fluorescence, of nasturtium (*Tropaeolum majus* L.), a species with dual ornamental and edible value. Plants were grown for four months under a 16-h photoperiod and a uniform photosynthetic photon flux density (118 ± 2 µmol m^{-2} s^{-1}). Treatments included monochromatic red (R) and blue (B) light, three red-blue combinations (R3B1: 75%R/25%B; R1B1: 50%R/50%B; R1B3: 25%R/75%B), and a cool white (W) LED control. Measured parameters included morphological traits, biomass accumulation, growth analysis indices, chlorophyll fluorescence, and light use efficiency (LUE). The results showed that monochromatic R light significantly promoted stem and root elongation and photosynthetic efficiency. Under the conditions of this study, the balanced R1B1 (1:1) treatment consistently yielded optimal results, producing the highest node number, total plant biomass (fresh and dry weight), and superior absolute and relative growth rates. The R1B3 treatment produced the largest leaf area, matching R1B1 in leaf fresh weight, while the red-dominant R3B1 treatment underperformed across most metrics. Photosynthetic performance analysis revealed no significant differences in the maximum quantum yield (Fv/Fm) or non-photochemical quenching (NPQ) among treatments. However, the effective quantum yield of PSII (ΦPSII) and photochemical quenching (qP) were highest under B and R1B1 light. Light use efficiency (LUE) peaked under R1B1 and white light, whereas R3B1 exhibited significantly reduced LUE. Overall, the results demonstrate that while red light selectively drives elongation, a balanced 1:1 red-to-blue spectrum delivers the most effective combination for integrated growth and physiological performance for optimizing biomass, growth, and photosynthetic performance in nasturtium cultivation, highlighting the importance of spectral optimization in horticultural lighting systems.

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1. Introduction

A key advantage of LED lighting is its precise spectral tunability, enabling the optimization of photosynthetic efficiency and plant morphology. By targeting specific photoreceptors, the light spectrum can be engineered to control physiological processes such as photomorphogenesis and secondary metabolite production. This allows for directed manipulation of plant architecture, nutritional quality, and growth cycles. Building on this spectral precision, LED technology has revolutionized horticultural lighting, playing a key role in controlled-environment agriculture, particularly for growing leafy vegetables. (Johkan *et al.*, 2010; Ouzounis *et al.*, 2015; Alrifai *et al.*, 2019; Pennisi *et al.*, 2019; Pennisi *et al.*, 2020). Plants exhibit varying photosynthetic performance and morphological responses when exposed to distinct regions of the light spectrum. A comprehensive analysis of these photobiological mechanisms enables the optimization of growth spectra, thereby improving agricultural productivity to meet growing global food demands (Bi *et al.*, 2024). Extensive research has investigated how light intensity, spectral composition, exposure duration, and source characteristics affect

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vegetable crop growth dynamics, photosynthetic performance, and phytochemical content (Kang *et al*., 2013; Kozai, 2016; Viršilė *et al*., 2017).

Among various plant species, nasturtium (*Tropaeolum majus* L.) has gained attention as a valuable ornamental-edible plant, appreciated both for its attractive appearance and medicinal properties/culinary uses (Bortolini *et al*., 2022). The vertical farming paradigm has been conceptualized as an innovative solution to address land scarcity by utilizing vertical space through multi-level cultivation structures (Petrovics and Giezen, 2022).

Vertical farming systems are designed to dramatically enhance agricultural output while minimizing ecological impact through urban-based, multilevel indoor facilities with precisely regulated growing conditions. Such facilities offer many potential advantages such as a clean, green food source, biosecurity, freedom from pests and droughts, and reduced reliance on transportation and fossil fuels (Benke and Tomkins, 2017). While market realities have prioritized profitable leafy greens, vertical farming's viability fundamentally hinges on the precise management of photosynthetic inputs, particularly artificial lighting (Avendaño-Abarca *et al*., 2022).

Modern vertical farms have largely transitioned from conventional lighting systems to advanced LED technologies, which offer numerous advantages, including spectral tunability to match plant photoreceptor requirements, significantly improved energy efficiency, and extended operational lifespan (Trivellini *et al*., 2023). These characteristics make LEDs particularly suitable for controlled environment agriculture, where energy inputs represent a major operational cost. The tunable spectral output of LED systems enables precise alignment with plant photoreceptive pigments, facilitating optimized growth while simultaneously modifying structural development and biochemical processes (Massa *et al*., 2008; Cocetta *et al*., 2017).

Unlike traditional high-pressure sodium (HPS) lamps, LED technology offers a more energy-efficient solution for enhancing greenhouse lighting. By supplementing natural sunlight, LEDs help extend the growing season and improve vegetable yields, enabling better optimization of production (Carney *et al*., 2015). Modern lighting systems enable horticulturists to achieve multiple cultivation benefits, including controlled flowering timing, year-round harvests, uniform crop yields, optimized plant architecture through root and stem development management, precise pigment regulation in foliage and blooms, and overall quality enhancement (Azizi *et al*., 2024).

Studies indicate that modifying the light spectrum can profoundly affect plant physiology, impacting traits such as growth rate, biomass accumulation, and leaf and root development (Kim *et al*., 2024; Shin *et al*., 2024). Research by Hirai et al. (2005) demonstrated that the spectral composition of visible light , mediated by photoreceptors such as phytochromes for red and cryptochromes for blue, significantly affects internode elongation, a crucial factor governing overall plant height, with observed variations in response patterns across different species. Saito et al. (2010) observed that lettuce plants exposed solely to red light exhibited enhanced photosynthetic activity, increased leaf production, and higher fresh biomass accumulation relative to those grown under blue light or a combination of red and blue (R1B1) light wavelengths. According to the findings of Su and Hyeon (2014), spectral wavelengths of 634 nm and 659 nm in red light, along with 450 nm in blue light, were identified as the most effective for enhancing photosynthetic rates. These specific light wavelengths were shown to improve key growth parameters, including leaf area, shoot fresh weight, leaf chlorophyll content, and anthocyanin levels in plants.

Wang et al. (2022a) demonstrated that the CAM plant *Ottelia alismoides* exhibited increased leaf size under blue LED light but a decrease under red LED light. Research by Cope et al. (2014) demonstrated that exposure to blue spectral radiation suppresses hypocotyl extension in lettuce (*Lactuca sativa*) and radish (*Raphanus sativus*), while simultaneously stimulating lamina expansion. Photobiological research has established that targeted spectral tuning, such as supplemental UV-A LED irradiation under varying light quality and photoperiod, enables precise manipulation of plant growth parameters. Building upon the foundational work of Su and Hyeon (2014) on photomorphogenesis, Shin et al. (2024) on secondary metabolism, and Davis and Burns (2016) on light-use efficiency, our results confirm that wavelength-specific responses can be harnessed to optimize plant development. These cumulative findings provide a scientific basis for developing customized lighting regimes tailored to specific crop requirements in controlled environment agriculture.

Light-mediated effects span a comprehensive range of developmental processes in plants, encompassing the enhancement of photosynthetic efficiency, the regulation of architectural patterning during morphogenesis, and the precise manipulation of reproductive timing (Mitchell *et al.*, 2015), or enhancing antioxidant activity (Ramalho *et al*., 2002) or were shown in Pansy (*Viola* × *wittrockiana Rose*), highest foliage fresh and dry weights, stem diameter, leaf area, and leaf number in plants exposed to 85% R: 15% B. and cultivation under pure red spectrum lighting produced optimal results for root biomass parameters (both fresh and dry weight) and stem elongation, surpassing the performance of plants grown under alternative light spectra (Rashidi *et al*., 2018).

This study aims to address this research gap by investigating the physiological and morphological responses of *T. majus* to varying ratios of blue light within a controlled LED lighting environment. Specifically, the experiment will assess five distinct spectral treatments over a four-month cultivation period: monochromatic red (R), mixed red 75% plus blue 25% (R3:B1), balanced red 50% plus blue 50% (R1B1), mixed red 25% plus blue 75% (R1:B3), and monochromatic blue (B). The findings of this research will provide critical insights into how *T. majus* adapts to different light conditions, offering practical guidance for commercial growers seeking to enhance yield and quality in controlled-environment agriculture. Additionally, the results could contribute to the advancement of vertical farming systems, where efficient light management is crucial for maximizing productivity in space-limited settings. By identifying the most effective spectral composition for nasturtium growth, this study may also support broader applications in ornamental horticulture and edible flower production, where this species is valued for both its aesthetic and nutritional properties.

2. Materials and methods

Nasturtium seeds from Hem Zaden B. V were surface-disinfected and pre-soaked before planting in 10 × 10 cm (diameter × height) pots containing 1:1 (v/v) mixture of perlite: cocopeat substrate (0.875 L). Plants were grown in six environmentally controlled chambers under different light treatments: monochromatic red (650 nm, 2-2.8 W), and blue (465 nm, 2.8-3.4 W/diode) provided by Shenzhen Getian Opto-electronics), three R: B ratios R3B1 (R75%B25%), R1B3 (R25%B75%), R1B1 (R50%B50%) and cool-white LED control (Dulux T PLUS 26W/840, Osram). All lighting systems were suspended 150-250 mm above the plants, providing a PPFD of 118±2 μmol m^{-2} s^{-1} for 16 h daily, as verified using an LI-250A PAR sensor (LI-COR). The specifications for lighting system are given in Fig. 1 and Table 1. Growth chambers were set to 24±2°C (day) and 20±2°C (night) and RH of 70±10%. Plants were irrigated as needed, and 1/2 strength Hoagland nutrient solution in the amount of 100 mL is given to the plants once a week. The solution EC was adjusted (1±0.2 dS m^{-1}) and pH (6 and 6.5).

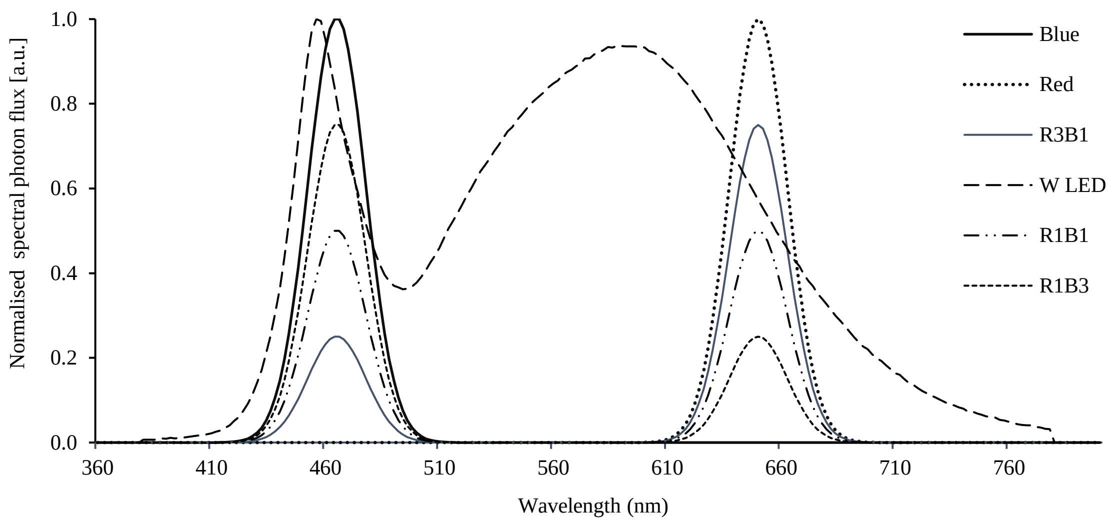

Figure 1. Normalized spectral photon flux distribution of the light treatments.

Table 1 Photosynthetic photon flux density (PPFD), B and R spectrum proportion plus duration of lighting cycles for each lighting system

Lighting systems	PPFD (μmol m^{-2} s^{-1})	R (%)	B (%)	Lighting cycle (h day^{-1})
R	118±2	100	0	16
R3B1	118±2	75	25	16
R1B1	118±2	50	50	16
R1B3	118±2	25	75	16
B	118±2	0	100	16
W	118±2	NA	NA	16

R: Red; B: Blue; W: Cool White

2.1. Measurements

2.1.1. Growth parameters

Plants were assessed biweekly to measure growth characteristics, including stem length, leaf number, and node number. Plants were grown for 4 months then After 30, 60, and 120 days from the beginning of the experiment, plants from each treatment and control were carefully uprooted and weighed to determine fresh weight (stem, leaf, and root), the leaf area was measured using a leaf area meter (Li 3100C). All fresh organs were then oven-dried at 80°C for 72 hours to determine dry weight. Growth analysis variables were estimated for 3 time stages according to Radford (1967) and Hunt et al. (2002). These variables included Absolute Growth Rate (AGR, g day^{-1}), relative growth rate (RGR $gg^{-1}day$), net assimilation rate (NAR DW g $m^{-2}d^{-1}$) and leaf area ratio (LAR cm^2g^{-1}), leaf weight ratio (LWR m^2 gg^{-1}), stem weight ratio (SWR m^2 gg^{-1}) and root weight ratio (RWR m^2 gg^{-1}) during the treatments (30-60 days; 60-90 days; 90-120 days). These variables were calculated using Equations 1–7.

(1) $AGR = W_2 - W_1/t_2 - t_1$

(2) $RGR = \ln W_2 - \ln W_1/t_2 - t_1 = NAR \times LAR$

(3) $NAR = W_2 - W_1/LA_2 - LA_1 \times \ln LA_2 - \ln LA_1/t_2 - t_1$

(4) $LAR = LA_2 - LA_1/\ln LA_2 - \ln LA_1 \times \ln W_2 - \ln W_1/W_2 - W_1$

(5) $LWR = L_2 - L_1/\ln L_2 - \ln L_1 \times \ln W_2 - \ln W_1/W_2 - W_1$

(6) $SWR = S_2 - S_1/\ln S_2 - \ln S_1 \times \ln W_2 - \ln W_1/W_2 - W_1$

(7) $RWR = R_2 - R_1/\ln R_2 - \ln R_1 \times \ln W_2 - \ln W_1/W_2 - W_1$

2.1.2. Chlorophyll fluorescence properties

At the end of the experiment, four leaves were randomly selected from different plants per treatment. The leaves were then dark-adapted for 20 min, before being subjected to chlorophyll fluorescence measurements Handypea, Hansatech, UK (Chl Fluorescence meter). Using a leaf clip, minimal dark-adapted fluorescence (F_0) was recorded, followed by a saturating pulse (<3500 μmol m^{-2} s^{-1}, 100 s) to determine maximal dark-adapted fluorescence (Fm). The maximum quantum yield of PSII (Fv/Fm) was derived as (Fm – F_0)/Fm. The same leaves were then subjected to actinic light (120 μmol m^{-2} s^{-1}, 100 s) for 100 s. light-adapted maximal fluorescence (Fm') was measured. From these values, effective quantum yield (ΦPSII), photochemical quenching (qP), and non-photochemical quenching (NPQ) were calculated (Table 2).

2.1.3. Light use efficiency (LUE):

Light use efficiency (LUE) was calculated as LUE = ΔW / PAR, where ΔW is the increase in whole-plant dry weight (g m^{-2} h^{-1}) and PAR is the photosynthetically active radiation absorbed by the plant (μmol m^{-2} h^{-1}).

Table 2. Measured components of chlorophyll fluorescence and their equations

Equation	Description	Parameter (Symbol)
fm-fo	Variable fluorescence	fv
(fm-fo)/fm	Maximum photochemical quantum yield of photosystem II	fv/fm
(Fm′–F′)/Fm′	The effective photochemical quantum yield of photosystem II	PhiPSII or ϕPSII
(Fm′–F′)/(Fm′–Fo′)	Photochemical quenching	qp
(Fm–Fm′)/Fm′	Non-photochemical quenching	NPQ or qn

2.2. Statistical analyses

One-way ANOVA was used for analyzing characteristics measured at the end of the experiments, but the measurements recorded over the months were subjected to two-way ANOVA to determine the effect of light quality, time, and their interactions on the measured morphological traits. All the statistical analyses, including ANOVA and mean comparison, applying Tukey's honestly significant difference (HSD) test ($P \leqslant 0.05$), were performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Growth and morphological characteristics

Our analysis revealed significant light-treatment effects (p<0.01) on all measured growth parameters, including vegetative and reproductive characteristics. Monochromatic red (R) light proved particularly influential, inducing markedly greater stem and root elongation compared to other spectral regimes. While R was significantly different from all other treatments, we observed a clear inhibitory trend: increasing blue (B) light proportions progressively suppressed elongation rates in both stems and roots (Figs. 2A–2D).

Our results demonstrate significant variations in nasturtium growth parameters across different light spectra. The R1B3 treatment produced the largest leaf area (Fig. 2B), while the balanced R1B1 spectrum yielded optimal values for multiple growth metrics including node number (Fig. 2C), stem biomass (both fresh and dry weight) (Figs. 3B, 3G), one of the highest root biomass values (fresh and dry weight) (Figs. 3C, 3E) and total plant biomass (both fresh and dry weight) (Figs. 3F, 3H).

Interestingly, leaf fresh weight showed comparable results under both R1B1 and R1B3 conditions, with no statistically significant difference between these treatments (Fig. 3A). Conversely, the R3B1 treatment consistently produced the lowest values across most measured parameters, including leaf area (Fig. 2B), fresh/dry weights of stems (Figs. 3B, 3G), leaves (Figs. 3A, 3D), roots (Figs. 3C, 3E), and total biomass (both fresh and dry) (Figs. 3F, 3H).

Monochromatic blue light exhibited notable performance, ranking second only to R1B3 for leaf area development (Fig. 2B). In the number of node, it followed the R1B1 treatment (Fig. 2C), while for leaf fresh weight, it performed comparably to both R1B1 and R1B3 treatments, showing no significant difference from white light controls (Fig. 3A). Furthermore, blue-light-grown plants showed secondary prominence in stem fresh weight, root fresh weight, leaf dry weight, and stem dry weight after the R1B1 treatment (Figs. 3B–3D, 3G).

The results indicate that monochromatic red (R) light enhances plant growth, particularly stem and root development, while blue (B) light may inhibit elongation (Figs. 2A, 2D).

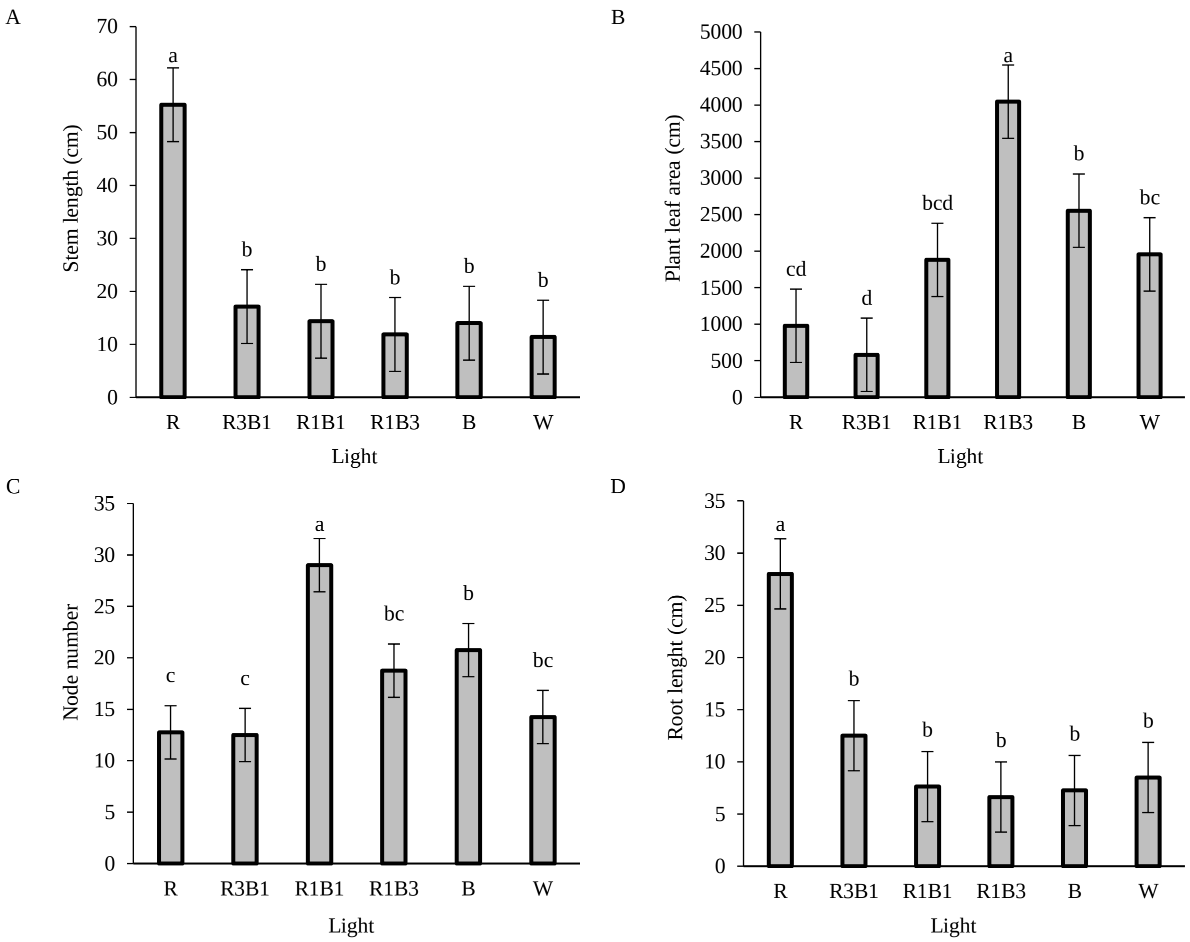

Figure 2. Different light treatments altered nasturtium growth and structure, affecting stem and root length (A, D), plant leaf area (B), and node number (C). Light sources included: Pure red (R) or blue (B) LEDs, Mixed ratios (R3B1, R1B1, and R1B3), and White LED (W) as control. Bars with the same letter are not significantly different at *p*⩽0.05, according to Tukey's honestly significance test.

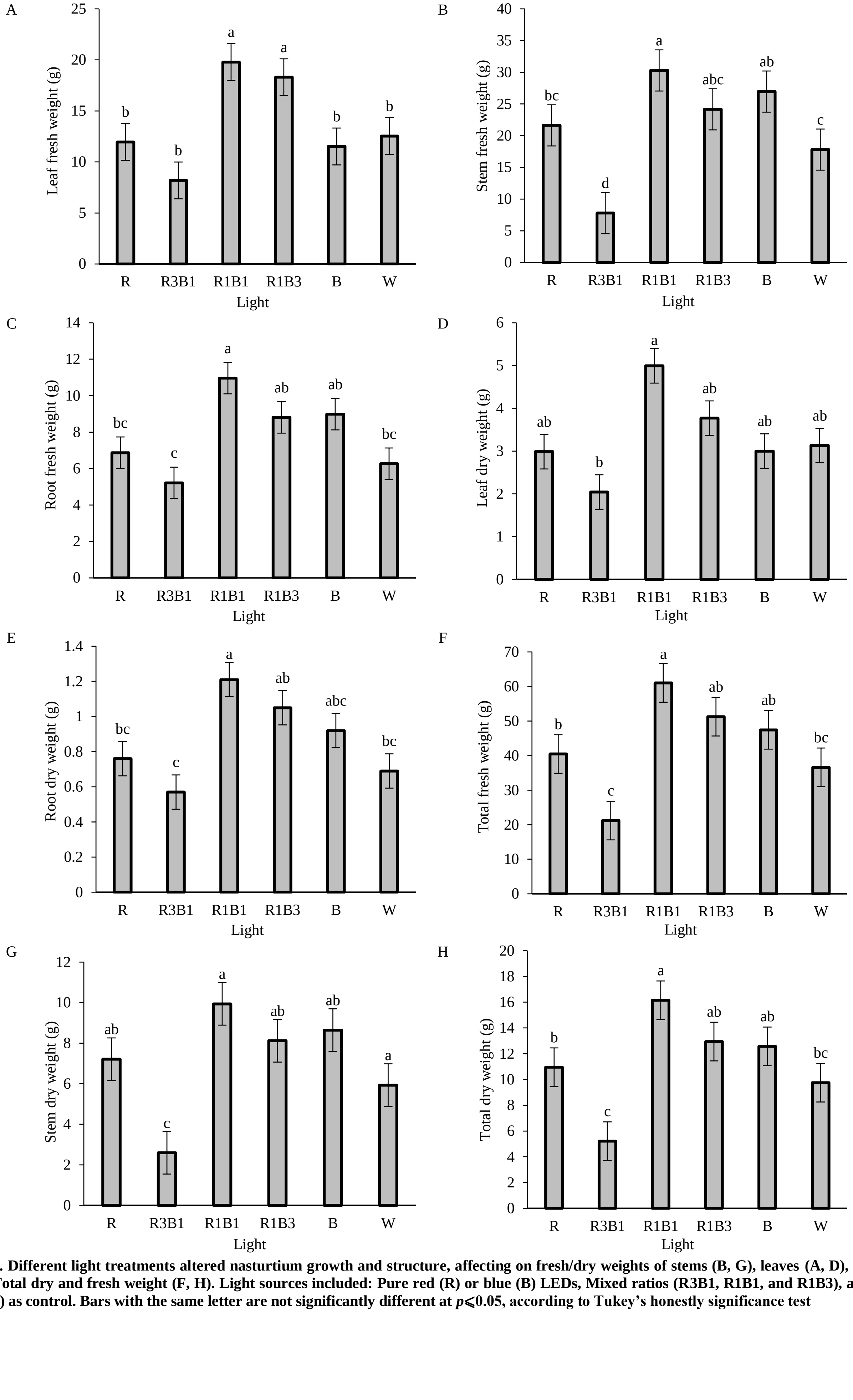

Figure 3. Different light treatments altered nasturtium growth and structure, affecting on fresh/dry weights of stems (B, G), leaves (A, D), and roots (C, E). Total dry and fresh weight (F, H). Light sources included: Pure red (R) or blue (B) LEDs, Mixed ratios (R3B1, R1B1, and R1B3), and White LED (W) as control. Bars with the same letter are not significantly different at $p\leqslant 0.05$, according to Tukey's honestly significance test

The highest root and stem lengths were observed under R treatment, suggesting red light (600–700 nm) promotes elongation, whereas blue light (400–500 nm) increases compactness and leaf thickness. Supporting these findings, Nissim-Levi et al. (2019) found that blue light induced denser growth and more flowers in *Chrysanthemum morifolium*, while red light accelerated flowering and stem elongation. Blue light likely inhibits elongation via cryptochrome activation (Liu *et al*., 2016). Both (R and B) light positively affected node number and leaf area, but B light's effect was threefold stronger (Figs. 2B, 2C). However, these lights alone had limited impact.

Contrary to our data, Izzo et al. (2019) reported that red light alone produced smaller leaf area and dry weight in *Atriplex hortensis* compared to blue or red-blue combinations. Wang et al. (2016) observed that lettuce (*Lactuca sativa* L.) under red (R) light showed no reduction in leaf area or number compared to R/B combinations. Plants adapt to light quality by altering leaf morphology (e.g., size, shape) or photosynthesis (e.g., Rubisco synthesis) (Senger and Bauer, 1987). Wollaeger and Eric (2015) found similar leaf numbers in impatiens, salvia, petunia, and tomato across blue (B) and R treatments. Conversely, He et al. (2015) reported that Brassica alboglabra under R+B LEDs had more leaves and larger leaf area than R alone, though excessive (B) light damaged PS II. The observed increase in stem length under red (R) light was accompanied by a reduction in node number, suggesting that R light promotes internode elongation, thereby increasing the spacing between nodes.

In contrast, blue (B) light and its dominant combinations (e.g., R1B1 and R1B3) induced stem compression—resulting in shorter stems but a higher node count per unit length (Figs. 2A, 2C). This inverse relationship between stem elongation and node density implies that these responses may be independent of physiological effects rather than causally linked. Specifically, while (B) light restricts internodal spacing, leading to compact growth, R light favors internode extension, reducing node density without necessarily suppressing node initiation. Our results showed that combined R1B1 treatment yielded among the highest values for all biomass parameters (Fig. 3). Increasing the proportion of either blue (R1B3) or red (R3B1) light, or using them separately, reduced these measurements, with blue light showing greater influence. Supporting this, Wollaeger and Runkle (2014) found tomato and sage shoot dry weight increased by 48-112% under red light versus red+blue combinations. Similarly, Ohashi-Kaneko et al. (2007) reported lettuce and tomato shoot dry weight was 14-52% higher under red light compared to blue or mixed spectra.

Combined red-blue light maximized plant weight, suggesting synergistic growth benefits, while blue light alone was less effective. In Cymbidium orchids, 25%R+75%B yielded higher fresh weight than monochromatic treatments (Tanaka *et al*., 1998). Petunia under R160 showed 84% greater fresh weight than R80+B80, though tomato weights remained similar across treatments (Wollaeger and Eric, 2015). Contrastingly, *Centella asiatica* exhibited no weight differences between light intensities, but higher red proportions increased fresh weight (Song *et al*., 2022), aligning with cucumber/tomato data (Liang *et al*., 2021). Roses under 20%B+80%R had higher leaf dry weight but reduced biomass versus HPS lamps (Terfa *et al*., 2012; Terfa *et al*., 2013). In this study, no significant difference in maximum leaf fresh weight was observed between R1B1 and R1B3 treatments (Fig. 3A). Blue light significantly influenced stem fresh/dry weight (Figs. 3B, 3G) and plant leaf area (Fig. 2B). In Pak Choi, 23% blue light yielded the highest stem biomass and leaf area, followed by 27% and 100% blue; monochromatic red light showed the lowest values (Bian *et al*., 2017).

The blue-red combination critically affects growth morphology, pigmentation, and light absorption efficiency, though optimal ratios vary by species and growth stage (Li and Kubota, 2009). Cucumber seedlings under 2R1B LEDs exhibited stouter growth, shorter hypocotyls, and higher dry weight versus other ratios (Song *et al*., 2019). Monochromatic blue (B) light significantly increased leaf area, with R1B3 showing the strongest effect (Fig. 2B), underscoring blue light's role in leaf expansion when combined minimally with red. Conversely, African violets under red (R) light developed larger leaves than under B or R1B1 spectra (Ahmadi *et al*., 2024). Notably, replacing 25% R with B in R1B1 treatments significantly reduced leaf size, demonstrating blue light's regulatory role in leaf expansion. The optimal spectral balance remains species-dependent. Light quality significantly influenced plant characteristics, with red and fluorescent light producing larger leaves than blue light (Park *et al*., 2024). However, replacing 25% red with blue light eliminated this difference (Fig. 2B). Similar effects were observed in other species: poinsettias under 80% B/20% R showed reduced height and leaf area (Islam *et al*., 2012), while annual flowers with 30% blue light had shorter stems (Randall and Lopez, 2014). Roses and chrysanthemums exhibited decreased height with increasing blue light (20-100%), unlike *Campanula* (Ouzounis *et al*., 2014). Lettuce seedlings showed suppressed elongation under red light when supplemented with ≥15 µmol m^{-2} s^{-1} blue (Hoenecke *et al*., 1992). Michaelmas daisy roots were longest under blue/green/white light but shortest under 25% B/75% R (Schroeter-Zakrzewska and Kleiber, 2014). Gerbera displayed reduced shoot weight under red light but increased root weight under blue/white light (Gabryszewska and Rudnicki, 1995).

3.2. Growth analysis parameters

The studied light treatments, time, and interaction of light on time significantly affected the characteristics of AGR, RGR, NAR, LAR, LWR, SWR, and RWR. At the statistical level of 0.01 (Table 3). The analysis of growth parameters revealed distinct patterns across different light treatments (Table 4). As shown in Fig. 4A, the R1B1 treatment demonstrated superior performance in absolute growth rate (AGR), particularly during the 90-120 day period, followed closely by its 30-60 day results. In contrast, the R3B1 treatment consistently showed the lowest AGR values during both 30-60 and 60-90 day intervals (Table 4).

Table 3. Analysis of growth variances in the studied light treatments in three time periods

S. O. V	df	Mean squares						
		AGR	RGR	NAR	LAR	LWR	SWR	RWR
Time (T)	2	0.357**	0.002**	3.422**	4080.046**	0.252**	0.070**	0.031**
Light (L)	5	0.276**	0.000**	2.145**	1671.237**	0.022**	0.046**	0.013**
T × L	10	0.033**	0.000**	1.631**	178.326**	178.326**	0.0002**	0.003**
Residuals	54	0.007	8.857	1.397	23.518	0.000	0.000	3.112
Total	72	-	-	-	-	-	-	-
CV (%)		22.67	24.14	31.97	20.23	6.73	2.55	2.94

In the sources of variation, Time (indicating time:30-60, 60-90, 90-120 days), Light (treatment of different light levels), Time*Light (interaction of light on time), CV coefficient of variation, absolute growth rate (AGR), relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), leaf weight ratio (LWR), stem weight ratio (SWR), root weight ratio (RWR). **Significant at 1% of probability level, * Significant at 5% of probability level, ns non-significant.

Table 4. Effect of different light spectra on Growth parameters (AGR, RGR, NAR, LAR, LWR, SWR, and RWR) 3 times in nasturtium. Data are presented as means. Values in a column with the same letter are not significantly different at $p\leqslant 0.05$, according to Tukey's honestly significance test

Light quality	Time (day)	AGR (g day^{-1})	RGR (g g^{-1} day)	NAR (g m^2 day^{-1})	LAR (cm^2 g^{-1})	LWR (g g^{-1})	SWR (g g^{-1})	RWR (g g^{-1})
	1	0.2550bcd	0.0150bc	30.50ab	4.9075e	0.2475def	0.4025fgh	0.3025a
R	2	0.2250cd	0.0100c	7.50cd	13.315de	0.2975bcde	0.5350abc	0.1700ef
	3	0.4525abcd	0.0100c	7.50cd	18.980de	0.1325gh	0.5350abc	0.1700ef
	1	0.0925d	0.0125bc	16.75bcd	6.0675e	0.3800ab	0.3150i	0.3000ab
R3B1	2	0.1400d	0.0125bc	8.00cd	14.415de	0.3975a	0.3525hi	0.2525cd
	3	0.2375bcd	0.0100c	7.75cd	19.945cde	0.0925h	0.3700hi	0.2500cd
	1	0.6750a	0.0375a	44.50a	8.2025de	0.3400abc	0.3925gh	0.2425cd
R1B1	2	0.3425abcd	0.0100c	5.00cd	18.112de	0.3200abcd	0.5000bcd	0.1800ef
	3	0.6775a	0.0100c	5.00cd	25.420bcde	0.2200efg	0.5000bcd	0.1800ef
	1	0.6425ab	0.0475a	26.00b	14.415de	0.3850ab	0.3500hi	0.2325d
R1B3	2	0.2850abcd	0.0100c	2.00d	45.497ab	0.3550ab	0.4725de	0.1700ef
	3	0.5700abc	0.0100c	2.00d	65.182a	0.2025fg	0.4700de	0.1700ef
	1	0.4675abcd	0.0300ab	22.00bc	13.617de	0.2525cdef	0.4575def	0.2675bc
B	2	0.2525bcd	0.0100c	3.00d	30.770bcd	0.2475def	0.5575ab	0.1950e
	3	0.5525abc	0.0125bc	3.00d	44.762ba	0.1350gh	0.5600a	0.1925e
	1	0.2300cd	0.0150bc	13.00bcd	10.900de	0.4000a	0.4350efg	0.1550f
W	2	0.2075cd	0.0100c	3.00d	30.282bcd	0.3375abc	0.4900cde	0.1700ef
	3	0.4075abcd	0.0100c	3.00d	43.090abc	0.1400gh	0.4900cde	0.1700ef

1: 30-60 days, 2: 60-90 days, 3: 90-120.days

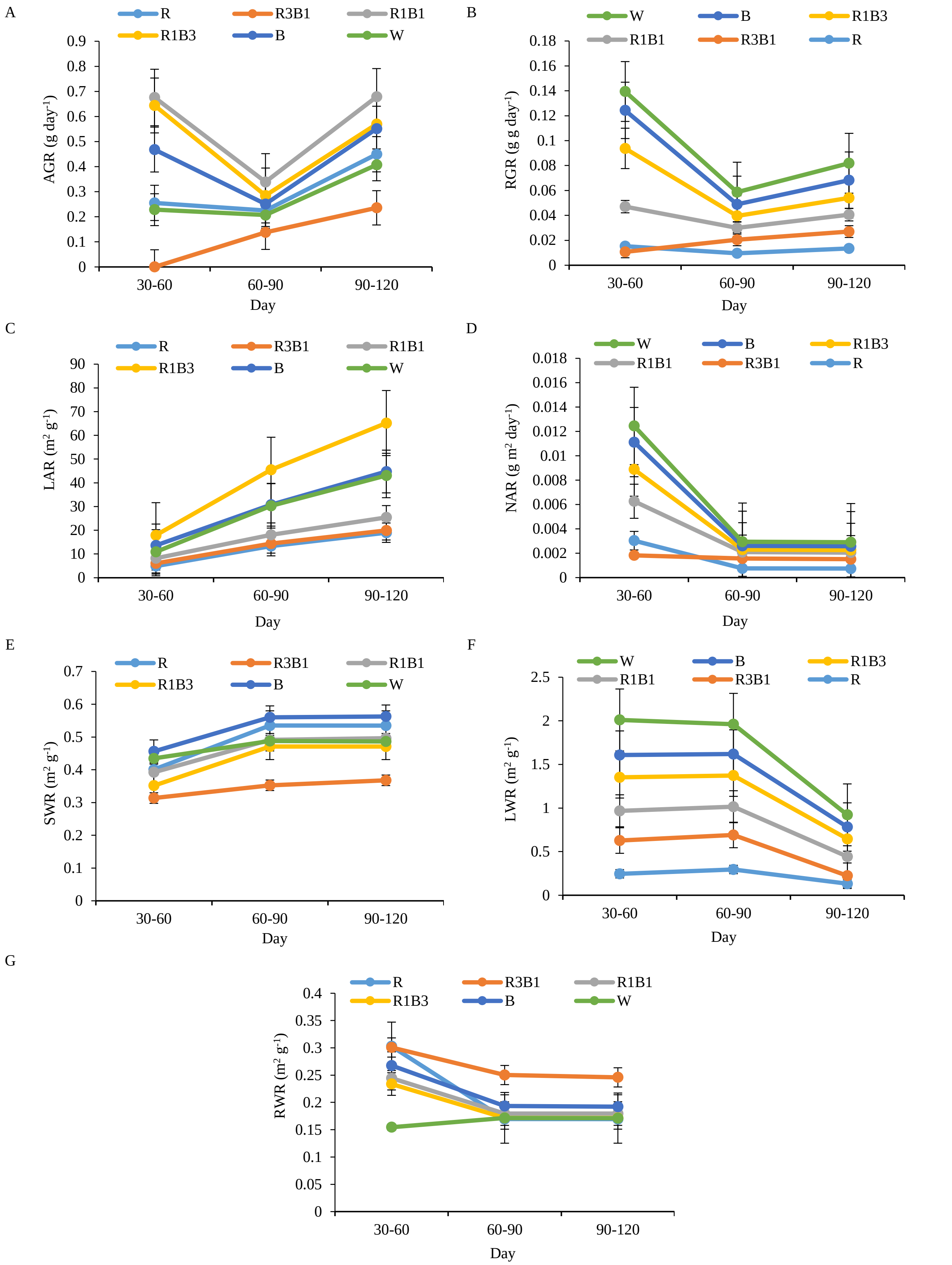

Figure 4. Changes of growth parameters by time in different light qualities: (R: 100% Red light, R3B1: 75% Red light+25% Blue, R1B1: 50% Red light+50% Blue light, R1B3: 25% Red light+75% Blue light, B: 100% Blue light, W: White light), Time (day): (30-60 days, 60-90 days, and 90-120.dayes).

When examining relative growth rate (RGR), Fig. 4B illustrates that the R1B3 and R1B1 treatments during 30-60 days produced the highest values, with other treatments showing statistically similar results. Net assimilation rate (NAR) was most favorable under R1B1 and R treatments during the early growth stage (30-60 days), as presented in Fig. 4D, while other light conditions yielded comparable values (Table 4).

For leaf area ratio (LAR), plants grown under R1B3 light during 90-120 days showed maximum values (Fig. 4C), followed by those under white and blue light treatments. The R and R3B1 treatments resulted in the lowest LAR measurements during the initial growth phase, as documented in Table 4. Further analysis of biomass allocation revealed that leaf weight ratio (LWR) peaked in the R1B3 treatment (Fig. 4F), while stem weight ratio (SWR) was highest under blue light during mid-to-late growth stages (60-120 days) (Fig. 4G). Root weight ratio (RWR) showed optimal development under R treatment during 30-60 days (Fig. 4G). The poorest performance metrics were observed in specific treatment combinations: R/R3B1 for LAR (Table 4), R3B1 for LWR (Fig. 4F), and white light for RWR during their respective measurement periods (Fig. 4G). These results, comprehensively presented in both Fig. 4 and Table 4, collectively demonstrate that the balanced red-blue (R1B1) treatment generally promoted the most favorable growth characteristics across multiple parameters, while treatments with imbalanced ratios (particularly R3B1) tended to produce suboptimal results. The data clearly show how specific light spectrum combinations at different growth stages influence nasturtium development, with detailed numerical comparisons available in Table 4 and visual representations in Fig. 4.

The results showed that by reducing the percentage of red light and replacing it with blue light (R1B3), the absolute growth rate (AGR) decreased, but the trend of decreasing the AGR was higher when the percentage of red light was twice as much as blue light (R3B1) (Fig. 4A). In this study by decreasing the percentage of blue light from 75% to 50%, the value of RGR decreased, but there was no significant difference, but in lower percentages, a significant difference was obtained in this index. Also, with the increase in the percentage of red light from 25% to 50%, the RGR increased to some extent, but when the percentage of red light reached 75%, this index began to decline (Fig. 4B).

This study revealed that blue light usually increases vegetative growth (leaves and stems), which can lead to an increase in plant dry weight (Fig. 4A) and, as a result, an increase in absolute growth rate (AGR). Increasing the percentage of blue light compared to red light decreased AGR because blue light inhibited the longitudinal growth of the stem and caused the stems to become shorter and thicker. However, because it did not reduce the leaf area, as a result, photosynthesis and plant growth were not limited (Fig. 4A). Balanced red-blue light optimized RGR, while monochromatic treatments were ineffective. RGR decreased with blue light reduction and showed a non-linear response to red light (peaking at 50%). Pure red light caused excessive stem elongation, reducing RGR. Temporally, RGR peaked at 30-60 days before declining and partial recovery (Table 4).

Blue light in appropriate concentrations (usually 20-30% of total light) can increase photosynthesis and biomass production, which directly affects AGR. Additionally, Blue light can increase RGR due to its positive effect on chlorophyll production and improving the photosynthesis process. Although blue light is essential for regulating processes like stomatal aperture, excessive exposure can reduce the Relative Growth Rate (RGR). A plausible hypothesis is that increased energy allocation to photo-protective and regulatory responses may limit resources for biomass accumulation. Confirming this mechanism requires future direct measurements of stomatal conductance and carbon partitioning (Fig. 4B).

High percentages of red light 100% longitudinal growth of stems is strongly stimulated because more energy is spent on longitudinal growth and leaf development is limited, thus reducing RGR (Fig. 4B). Red light Due to its key role in activating phytochromes and stimulating reproductive growth, red light can increase AGR in ornamental plants. Red light alone or in combination with Blue light increases the longitudinal growth of stems and biomass production However, Red light alone may cause excessive stem elongation with reduced biomass and excessive stem elongation, which can negatively affect plant quality (Fig. 4A).

Red light typically increases the Relative Growth Rate (RGR) because it provides more energy for photosynthesis and biomass production. Blue + Red Light Combination: This mix yields the best results for the Absolute Growth Rate (AGR), balancing vegetative and reproductive growth. It also enhances RGR since both wavelengths complement each other in photosynthesis and plant development. 75% Blue + 25% Red Light, significantly increases Leaf Area Ratio (LAR), but a higher percentage of blue light (100%) reduces this effect. White Light, as a balanced combination of red and blue light, it supports both vegetative growth (leaf expansion) and structural growth (stem and leaf thickness) (Fig. 4C). Red light stimulates vegetative growth and leaf expansion, which can lead to increased photosynthetic levels and improved NAR (Fig. 4D).

The R1B1 (red-blue) light combination during 30-60 days resulted in the highest net assimilation rate (NAR)**,** followed by red light (R) alone, suggesting that Red-blue synergy boosts photosynthetic efficiency in early growth stages. Red light alone maintains moderately high NAR but is less effective than combined spectra. Other light treatments had lower NAR, indicating limited benefits beyond these optimal combinations (Table 4). Blue light increases leaf weight ratio (LWR) but restricts stem elongation. Red light promotes stem growth and flowering but reduces LWR. White light balances these effects, favoring leaf dominance in early growth (Fig. 4F). As plants transition to reproductive stages, energy shifts to stems, flowers, and seeds, reducing LWR (Fig. 4F and Table 4). Early growth prioritizes leaf biomass for photosynthesis, while later stages favor reproductive structures, naturally lowering LWR. (90-120 days) induced measurable shifts in stem weight allocation compared to intermediate durations (60-90 days) (Table 4).

This duration-dependent response underscores the importance of considering both spectral composition and treatment timing in controlled environment agriculture. The results showed that the highest value of SWR index was obtained in (B) light treatment and then in (R) light treatment. There was no significant difference between R1B1 and W treatments. The lowest values of this index were created in R3B1 light treatment (Fig. 4E) and as seen in Fig. 4G, the highest value of RWR index was obtained in R3B1 light treatment and then simultaneously in R and Blight treatment. The lowest values of this index were created in W light treatment.

3.3. Photosynthetic and fluorescence of chlorophyll characteristics

In this study, the results showed that no significant changes were observed in the photosystem (II) maximum quantum efficiency in light environments with different ratio combinations (Fv/Fm). In other words, Fv/Fm values were almost similar in different light conditions and there was no significant difference between them. The results showed that the maximum quantum efficiency index of photosystem II (FV/FM) and NPQ, did not show significant difference in different light treatments (Table 5).

The results showed that the highest (FV/FM) is related to the combined treatments of 100% blue light and in the next step 25% red light + 75% blue light. The highest amount of the Quantum Efficiency of PSII (ϕPSII) was found in the treatment of 25% red light + 75% blue light, and there was no significant difference with the treatment of R1B1 light. The lowest value was obtained in the R treatment (Table 5). The highest photochemical quenching (qp) was obtained in the R1B1 light treatment, the lowest value was obtained in the (R100%) light treatment, and there was no difference between the other treatments also the highest Light Use Efficiency (LUE) was obtained in the R1B1 and W light treatments, the lowest value was obtained in the R3B1 light treatment, and there was no difference between B and R1B3 (Table 5).

Table 5. **Effect of different light spectra on photosynthetic and fluorescence of chlorophyll characteristics (Quantum efficiency of PSII (PhiPSII or ΦPSII), photochemical quenching (qp), nonphotochemical quenching (qn), maximum quantum efficiency of photosystem II (Fv/Fm) and light use efficiency (LUE)) in nasturtium.**

Light quality	PhiPSII	qp	qn	Fv/Fm	LUE
R	0.162^{b}	0.300^{b}	0.668^{a}	0.804^{a}	0.292^{bc}
R75%+B25%	0.244^{ab}	0.398^{ab}	0.636^{a}	0.812^{a}	0.160^{c}
R50%+B50%	0.294^{a}	0.548^{a}	0.732^{a}	0.812^{a}	0.528^{a}
R25%+B75%	0.302^{a}	0.504^{ab}	0.690^{a}	0.832^{a}	0.395^{ab}
B	0.284^{ab}	0.462^{ab}	0.698^{a}	0.836^{a}	0.467^{ab}
W	0.206^{ab}	0.356^{ab}	0.674^{a}	0.812^{a}	0.512^{a}

Data are presented as means. Values in a column with the same letter are not significantly different at $p \leqslant 0.05$, according to Tukey's honesty significance test.

Chlorophyll absorbs light, driving electron transport in Photosystem II (PSII)**,** which powers photosynthesis. This process can be measured via chlorophyll fluorescence (photochemical quenching), a key tool for assessing plant stress. The Fv/Fm ratio (typically 0.75–

0.85 in healthy plants) reflects the maximum photochemical efficiency of PSII, indicating how efficiently absorbed light is used for photosynthesis after dark adaptation. Fv/Fm serves as a proxy for photosynthetic capacity and overall plant health, revealing the condition of the photosynthetic machinery (Mathur *et al*., 2023). Analysis of chlorophyll fluorescence parameters showed that the R1B3 treatment, followed by the B treatment, improved the maximum photochemical efficiency of PSII (Fv/Fm), but overall, no significant differences were observed among light treatments (Table 5). This finding indicates that the performance of photosystem II was not affected by different light combinations and plants were able to maintain their photosynthetic efficiency at a similar level under different light conditions. These results can indicate the high adaptability of plants to environmental changes and their ability to maintain photosynthetic balance in diverse light conditions.

Considering that the time frame of our study is 4 months and the plant under investigation is the flower (*T. majus*), the results show that during this period, the changes in the Fv/Fm ratio in different light environments were not significantly different. This indicates that this flower has been able to adapt to different light conditions during these 4 months and maintain the performance of its photosystem II at a stable and favorable level which did not match the results of Yu et al. (2017) who reported the highest efficiency in red light. This result shows that these light compounds examined in stress-free levels can cause the rapid development of the photosynthesis apparatus and light protection mechanisms in the early stages of plant growth. The maximum photochemical quantum yield of PSII is widely accepted as a suitable index to evaluate the effect of environmental stresses on the photosynthetic apparatus (Su and Hyeon, 2014). Aliniaeifard and Seifi Kalhor (2017) also showed in the plant *Tradescantia virginiana* that increasing the ratio of blue to red light did not increase the level of light saturation point. Plants under blue light have lower chlorophyll levels compared to mixed light, leading to reduced photosynthesis and growth. This may be due to increased non-photochemical quenching (NPQ), dissipating excess light energy as heat. Studies (Zheng and Van Labeke, 2017) show that light quality affects Fv/Fm (max PSII efficiency): Red light (R) often lowers Fv/Fm. White (W) and red-blue (R1B1) light improve Fv/Fm. Blue light (B) can increase Fv/Fm in some species (e.g., Cordyline). Red-blue combinations (e.g., R10B90) enhance photosynthetic efficiency (ΦPSII, qP, ETR) compared to monochromatic light (Wang *et al*., 2022b).

Red-blue LED treatment significantly improves the electron transfer rate, the quantum efficiency (ΦPSII), the photochemical quenching (qP), and the efficiency of capturing excitation energy (Fv′/Fm′) in the open Photosystem II (PSII) reaction center (Xiaoying *et al*., 2012), whereas single light quality red and blue LEDs inhibits the PSII efficiency (Wang *et al*., 2009). Blue light (B) enhances photosynthesis and biomass production by improving the quantum efficiency of PSII (Fv/Fm and ΦPSII), leading to more efficient energy conversion. Red-blue combinations (R1B1) outperform red (R) or white (W) light, producing larger, more productive plants (Goins *et al*., 1997). Red light alone supports plant survival but yields lower biomass, while blue light boosts stomatal conductance (via increased density/index) without altering aperture size (Zheng and Van Labeke, 2017). R1B1 light showed the highest photochemical quenching (qP), while (R) light had the lowest (Table 5).

However, Luo et al. (2024) found that R9B1 (90% red, 10% blue) maximized qP, followed by R8B2, with R8B2 also yielding the highest Fv/Fm, Fv'/Fm', and ΦPSII. NPQ (non-photochemical quenching) remained similar across treatments, suggesting plants regulate heat dissipation equally under different wavelengths, including white light. The result is that While red-blue blends (R1B1) enhance photochemical efficiency, the optimal ratio varies (e.g., R9B1 or R8B2). NPQ adaptation ensures stable light stress responses regardless of spectrum. Plants efficiently adapt to white light, achieving high Light Use Efficiency (LUE), comparable to R1B1 (red-blue 1:1) treatment (Table 5). Though less absorbed than red light, blue light activates cryptochromes & phototropin's, enhancing stomatal opening, CO_2 uptake, and photomorphogenesis—boosting photosynthesis and LUE. Optimal Spectrum, Red-blue combinations (R1B1) maximize LUE by complementing each other—red drives chlorophyll absorption, while blue optimizes stomatal function and photoregulation. Balanced red-blue spectra (or white light) optimize LUE by synergizing light absorption and physiological responses.

4. Conclusion

This study demonstrated that the spectral composition of LED lighting significantly affects the vegetative growth and physiological performance of *Tropaeolum majus* L. Monochromatic red light enhanced stem and root elongation but led to lower overall biomass and photosynthetic efficiency. In contrast, the balanced red-blue (R1B1) treatment yielded the most favorable outcomes across growth parameters, chlorophyll fluorescence, and light use efficiency (LUE). Blue-enriched spectra (R1B3 and B) improved leaf expansion and ΦPSII, though they inhibited elongation. These findings highlight the critical role of spectral balance, with a 1:1 red-to-blue ratio emerging as the most effective strategy for optimizing biomass accumulation and photochemical performance in controlled environments. The consistent Fv/Fm and NPQ values across treatments indicate nasturtium's ability to maintain photoprotection and adapt to varying light spectra. Future research should address key gaps, including reproductive development, secondary metabolite production, and the interaction of light spectra with environmental stressors. Additionally, exploring dynamic lighting protocols tailored to specific growth stages could further enhance crop productivity in vertical farming systems.

Conflict of interests

All authors declare no conflict of interest.

Ethics approval and consent to participate

No humans or animals were used in the present research. The authors have adhered to ethical standards, including avoiding plagiarism, data fabrication, and double publication.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

Informed consent

The authors declare not to use any patients in this research.

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