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Research Article

Effect of different light spectra on morpho-physiological and biochemical characteristics of marigold (*Calendula officinalis* L.) under salinity stress

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Abstract

Utilizing complementary light spectra represents a potential novel approach for examining the enhancement of plant resilience amidst stressful conditions. The objective of this study was to explore the impact of various complementary light spectra on the growth and development of marigold plants under salinity-induced stress. The plants were cultivated in a greenhouse and exposed to blue, red, blue/red (2:1), blue/red (1:2), and white/yellow illumination throughout their growth stages. Stress conditions comprised control (non-stress) and salinity treatments (30, 60, and 90 mM NaCl). Salinity stress led to a reduction in fresh and dry weights, as well as leaf area index, while increasing proline content and Na concentration in roots and shoots. The combination of blue and red spectra caused superior stress mitigation compared to other spectra. Salinity stress reduced leaf chlorophyll and RWC, however, blue/red (2:1) treatment enhanced both parameters under NaCl stress. Salinity also increased the amount of total phenol content. White/yellow light exerted the most pronounced effect in reducing total phenol content at 90 mM salinity. Sodium uptake increased under stress, while potassium uptake decreased. The sentence was corrected. It can be concluded that the effects of salinity stress can be reduced by manipulating the supplemental light spectrum. The use of artificial light can be extended to stress.

Keywords: Ion Concentration, Leaf gas exchange, Light quality, Photosynthesis, Relative Water Content

Introduction

In recent years, the global agricultural landscape has faced increasing challenges due to environmental stresses, such as salinity, which significantly impact crop productivity and quality. Marigold, (*Calendula officinalis* L.), a well-known ornamental and medicinal plant, belongs to the daisy family. Marigold has garnered attention for its resilience and adaptability to various environmental conditions. However, its responses to salinity stress remain an area of critical investigation, particularly in the context of light spectra modulation (Verma *et al*., 2018).

The growth of plants is heavily influenced by various environmental factors, including light spectrum, temperature, watering, and nutrient levels (Kozai *et al*., 2016). These factors play a critical role in determining how plants grow, the amount they yield, and the accumulation of secondary compounds. Among these factors, light is a particularly significant abiotic element because it drives the process of photosynthesis, which generates internal chemical energy, and regulates various physiological responses in plants. Changes in light, including its spectral quality (ranging from 400 to 700 nm), intensity, and photoperiod, can trigger both primary and secondary responses in plants (Paradiso and Proietti, 2022).

Plants perceive not only light intensity and photoperiod but also light quality, including monochromatic and polychromatic light, as ambient growth environment signals that induce many physiological responses (Lauria *et al*., 2023). Manipulating spectral quality has a complex impact on plant physiology, morphology, and gene expression by initiating signals through photoreceptors such as phytochromes, phototropins, and cryptochromes (Wei *et al*., 2023).

Recently developed are horticultural Light Emitting Diodes (LEDs) modules, serving as artificial or supplementary grow lights. They hold promise for supplementing light in greenhouses and providing sole-source lighting in plant factory systems, enabling indoor plant cultivation under controlled environmental conditions (Aldarkazali *et al*., 2019). The subject of numerous recent studies has been the influence of LEDs on the growth, photosynthetic performance, morphology, yield, and anti-oxidant activity of various

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plant species (Zhen and Bugbee, 2020; Chutimanukul *et al*., 2022; Trivellini *et al*., 2023).

Salinity stress, arising from excessive salt accumulation in soil or irrigation water, poses significant challenges to plant growth by disrupting water and nutrient uptake, inducing osmotic stress, and triggering oxidative damage (Singh, 2022). Salinity stress disrupts the flow of electrons from reaction centers (RCs) to plastoquinone, affecting both the electron transfer chain's donor and acceptor sides. This interference hampers the electron transfer chain, leading to a decrease in photosynthesis efficiency (Al Hinai *et al*., 2022). Additionally, the accumulation of salt in mesophyll cells causes a reduction in carbon uptake and an increase in internal CO_2 concentration, resulting in reduced stomatal conductance. Among the factors that most significantly diminish plant photosynthesis is the closure of stomata, especially under moderate and high salinity conditions (Zahra *et al*., 2022).

This article aims to delve into the intricate interplay between light spectra modulation and salinity stress on the morpho-physiological and biochemical traits of marigold. We investigated the influence of five distinct light conditions on chlorophyll fluorescence and the plant's gas exchange parameters, focusing on marigold subjected to salinity stress. Salinity stress is known to impede plant growth, and extensive research has been dedicated to mitigating its adverse effects on plants. Consequently, we postulated that various light wavelengths could influence how plants respond to this stressor, potentially counterbalancing the disruptions in the functioning of the photosynthetic apparatus caused by stress. Such studies are crucial for analyzing plant responses to lighting systems under stressful conditions and for utilizing LEDs emitting various wavelengths as light sources in plant research and horticultural production. We hypothesize that different wavelengths of complementary light in greenhouse conditions reduce the negative effects of salinity stress by affecting the photosynthetic apparatus and vegetative growth, and ions uptake. This study aimed to investigate the effects of different light spectra on the growth and development of the marigold under salinity stress conditions. We expect that the results of this experiment will improve the functional properties of complementary light and optimize the lighting strategies for marigold plants.

Materials and methods

Plant material and growth conditions: In 2022, an experiment was carried out in the research greenhouse located at Lorestan University in Khorramabad, Iran, at coordinates 33.4647° N and 48.3390° E. The seeds of *Calendula officinalis* L. were obtained from a commercial seed supplier, Chia Tai Co. Ltd., situated in Bangkok, Thailand. These plants were cultivated in pots with a capacity of 1.3 liters, having dimensions of 135 mm at the top, 95 mm at the bottom diameter, and a height of 125 mm. The growth chamber provided an environment with ambient CO_2 levels, day/night temperatures of 22/20°C, and an average relative humidity of 65%. The experimental design involved subjecting the plants to five different light intensities and four stress levels, which included the following: A control group (without any stressors) and varying salinity levels (30, 60, and 90 mM NaCl). These salinity concentrations fall within the commonly accepted range for inducing salt stress in plants. The application of salinity stress treatments commenced 20 days after planting and persisted throughout the experiment until data collection was completed.

LED tubes with 24W of power, supplied by Parto Roshd Novin Company in Iran, were used. These LED tubes emitted light in various spectral ranges, including blue (B) with a peak at 460 nm, red (R) with a peak at 660 nm, blue/red ratios of 1:2 and 2:1, and white LEDs combined with yellow in a 1:1 ratio (400–700 nm) (Fig. 1). A spectrometer was used to draw these graphs. This device measures light intensity as a function of wavelength or frequency. The photon flux density (PPFD) was maintained at approximately 200±10 mmol m^{-2} s^{-1} for all treatments, and the photoperiod was set at 12 hours. The LED lighting systems were positioned 30 cm above each individual plant, with the LEDs oriented horizontally. The distance from the bench top to the base of the LEDs was 40 cm. Light intensity was regulated using a programmable controller. These LEDs were sourced from Iran Grow Light and possessed the following characteristics: A color rendering index (CRI) of 95%, 24 LEDs per system, and a light coverage area of 40 cm × 100 cm. Each treatment consisted of 10 pots, with each pot serving as an experimental unit containing three planted plants. Daily irrigation was provided to the plants. About two months after planting, the plants were harvested.

Plant growth and leaf area: Finally, plants were harvested and then their roots, shoots and flowers were carefully separated. To measure dry weight, the samples were placed in an oven at 70°C for 72 h, and then the dry weight of the samples was recorded. To measure leaf area, three leaf samples were randomly collected from each treatment, and leaf area was measured with 202 m-CI leaf area.

Leaf gas exchange: Plant gas exchange parameters include net CO_2 assimilation rate (A, μmol $CO_2 m^{-2} s^{-1}$), intrinsic water-use efficiency (WUEi, μmol CO_2 mol H_2O^{-1}), stomatal conductance (Gs, mol $H_2O m^{-2}$ s^{-1}), transpiration (E, mmol $H_2O m^{-2}$ s^{-1}), Sub-stomatal CO_2 concentration (Ci, μmol CO_2 mol^{-1}), and, instantaneous carboxylation efficiency (A/Ci) were measured using a portable photosynthesis system (LI-6800, LICOR Inc., Lincoln, NE) 50 days after planting. Around 9:00 AM and 12:00 AM, measurements were performed on completely expanded leaves.

Relative water content (RWC): RWC was measured to estimate the water status of the leaf samples, as described by Turner (Turner, 1981). In this study, a piece of the top collared leaf sampled for RWC measurement was approximately 2.5 cm × 5.0 cm and

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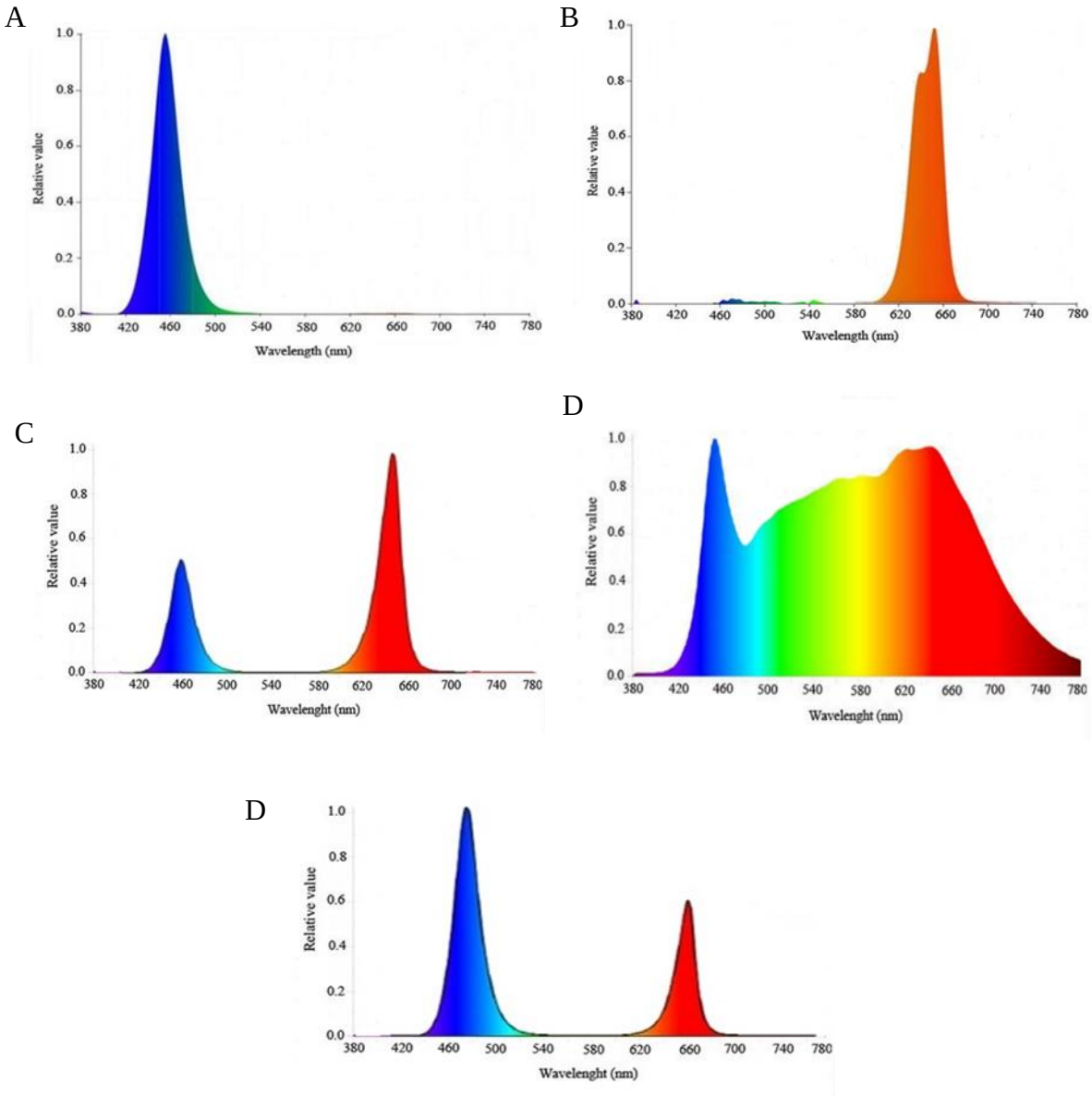

Figure 1. Relative distribution of different spectral LEDs. (A) monochromatic blue, (B) monochromatic red, (C) dichromatic blue/red (1:2), (D) dichromatic white/yellow (1:1), and dichromatic blue/red (2:1) used during the plant growth period.

weighed to obtain the fresh weight (FW). The samples were immediately submerged in deionized water overnight to ensure the tissue was fully turgid and weighed to obtain the turgid weight (TW). Finally, all samples were fully dried in a 60 °C dryer and weighed to obtain the dried weight (DW). These values were used to determine the RWC using the equation below.

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \times 100$$

Proline content: Proline was extracted from a sample of 0.5 g fresh leaf material samples in 3% (w/v) aqueous sulphosalycylic acid and estimated using the ninhydrin reagent according to the method of Bates *et al*. (1973). The absorbance of the fraction with toluene aspired from the liquid phase was read at a wavelength of 520 nm. Proline concentration was determined using a calibration curve and expressed as µmol proline g^{-1} FW.

Total phenolic compounds (TPC) and flavonoid content: According to protocols by Orsavova *et al*. (2019), total phenolic content (TPC) was established employing the Folin–Ciocalteu method. An aliquot of 400 µL extract was mixed with 2.0 mL of 10% F-C reagent and 1.60 mL 7.5% Na_2CO_3 solution. The mixture solution was shaken for 5 min and allowed to stand for 15 min at 37°C, followed by incubation in the dark for 1 h. The same T60 UV-Visible spectrophotometer was used to measure the absorbance at 725 nm to determine the TPC. Daily prepared standard calibration curves of gallic acid in methanol (20 to 250 µg/mL) were used to calculate the TPC.

Ttotal flavonoid content (TFC) applying $NaNO_2$, $AlCl_3 \cdot 6H_2O$, and NaOH using a UV/VIS spectrometer Lambda 25 (PerkinElmer, Waltham, MA, USA) (Orsavova *et al*., 2019). An aliquot of 0.40 mL extract (or standard) was mixed with 0.3 mL 5% $NaNO_2$ and 2.0 mL distilled water. The mixture solution was

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allowed to stand for 5 min, followed by the addition of 0.3 mL of 10% $AlCl_3$, and stood for another 6 min. After 1 min, the mixture solution was mixed with 2.0 mL 1.0 M NaOH and 3.2 mL distilled water, mixed with a vortex, and stood for 15 min at room temperature. The same T60 UV-Visible spectrophotometer was used to measure the absorbance at 422 nm to determine the TFC concentration. Daily prepared standard calibration curves of quercetin (20 to 600 µg/mL) were used to calculate the TFC.

Ion concentration: The determination of ion content was performed according to the methods of Munns *et al*. (2010). The sample was first baked at 105°C for 30 min and then dried at 70–80°C to a constant weight. After it was ground into powder, the fixed mass was weighed. After 30 ml of deionized water was added, the sample was shaken well and placed in a boiling water bath for 2 h. After cooling, the sample was filtered and diluted to 50 ml. The Na^+, and K^+ contents were determined by the atomic absorption method.

Experimental design and data analysis: This experiment was performed as a factorial with two factors in three replications as factorial and three single plants in pots. SAS software version 9.4 was used for data analysis (SAS Institute, Cary, NC, USA). https://www.sas.com/en_us/home.html). All data were statistically analyzed using a two-way ANOVA model. By observing significant treatment effects in the analysis of variance (ANOVA), significant mean differences ($P<0.05$) were calculated using the multiple ranges Duncan test as a post hoc. Once the differences between the means are demonstrated, it is possible to determine which means are different using post hoc range tests and pairwise multiple comparisons. Range tests identify homogeneous subsets of means that are not different from each other. Principal component analysis and biplots were performed using XLSTAT software version 2015 (https://www.xlstat.com /en/news/version-2015-6). A correlation plot was drawn with Origin Pro software version 2021 (https://www.originlab.com/2021). The graphs were drawn using microsoft excel (2016).

Results

The results showed that light, stress, and their interaction had significant efects on vegetative traits (Table 1). Salinity stress significantly reduces the fresh weight and dry weight of plants, leaf area and plant height under different light conditions. Under 90 mM salinity stress, Blue/Red (1:2) light caused the least reduction in root fresh weight (−19.71%), compared to the non-stressed plants. Blue light had the greatest effects on leaf area index in salinity stress. The highest leaf area values were observed in control and plants under blue/red (2:1) light treatments. Under 90 mM salinity stress, the lowest leaf area index (1.35) was observed in plants supplied with white/yellow light. The highest fresh weight of the shoot (78.3±1.04) was in the control treatment (no salt stress) and related to white/yellow light. Also, its lowest value (40.6±1.65) was in plants under 90 mM NaCl treatment in blue light treatment. The highest dry weight of shoot (17.23±0.53) in the control treatment (no salinity stress) was related to Blue/Red (1:2) light, and the lowest (13.42±0.36) in 90 mM salinity stress was in the blue light treatment.

According to ANOVA, plant gas exchange parameters were significantly affected by different light spectra, stress, and their interaction effects. Net CO_2 assimilation (A) of plants was influenced considerably by salinity stress and different light spectra. Net CO_2 assimilation decreased under salinity stress compared to the control. Under salinity stress, blue/red (1:2 and 2:1) light had the greatest effect on increasing CO_2 assimilation and had the lowest percentage of reduction compared to the control treatment (Table 2).

Transpiration rate (E) and stomatal conductance (Gs) decreased under salinity stress, the highest amount of transpiration was observed in the control plants and was associated with red light, while the lowest amount was recorded under 90 mM stress related to blue/red light at a 1:2 ratio. Salinity stress reduced water use efficiency (WUEi) compared to the control treatment. Under all stress conditions, blue light significantly increased WUEi. The lowest WUEi was observed in the Blue/Red (1:2) treatment.

According to the results (Table 2), light and stress had a significant effect on SPAD index. The results showed that the SPAD index was highest under white/yellow and decreased under salt stress conditions. Blue/Red (2:1) light had the greatest effect in increasing SPAD index. For other light spectrums, the SPAD index decreased under stress conditions. Also, the lowest SPAD index was observed under salinity stress (90 mM) treatment.

According to the results (Fig. 2), light and stress had a significant effect on RWC content. The results showed that the RWC content was highest under blue/red and red light and decreased under salt stress conditions. The lowest level of effect in reducing salinity stress was related to white and yellow light (Fig 2).

Leaf proline content was significantly influenced by light spectra ($P<0.05$) and salinity stress ($P<0.01$). Furthermore, the interaction effect of these two factors was also significant at a five percent probability level. Salt stress caused a significant increase in proline content. The highest proline level (11.4 µmol Fw^{-1}) was observed under 90 mM stress in red light, while the lowest level (0.95 µmol Fw^{-1}) was observed in the control treatment under white/yellow light (Fig. 3).

Figure 4 shows that light and stress have a significant effect on root and shoot elements concentrations. Salinity stress increased Na content in shoot and root. The highest root Na concentration was observed in the 90 mM salinity level and white/yellow light treatment (Fig 4a). The lowest Na concentration in shoot and root were observed under a blue/red 1:2 ratio in the control treatment (Fig 4b). Plants grown under white/yellow light had the lowest concentration of root

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Table 1. Interaction effect of light spectra and salinity stress on growth characteristics of marigold.

Light sources	Stress	Root fresh weight (g plant^{-1})	shoot fresh weight (g plant^{-1})	shoot dry weight (g plant^{-1})	Leaf area index	Plant height (cm)
Blue	Control	7.5 ± 0.45 ^{d}	60.3 ± 2.5 ^{b}	14.92 ± 0.62 ^{d}	2.86 ± 0.12 ^{c}	17.2 ± 1.02 ^{ab}
	30 mM	7.2 ± 0.41 ^{d}	58.6 ± 3.22 ^{bc}	14.88 ± 0.51 ^{de}	2.74 ± 0.09 ^{cd}	15.2 ± 0.25 ^{bc}
	60 mM	6.8 ± 0.37 ^{de}	53.5 ± 1.36 ^{bc}	14.59 ± 0.24 ^{e}	2.26 ± 0.1 ^{e}	11 ± 0.29 ^{ef}
	90 mM	5 ± 0.37 ^{g}	40.6 ± 1.65 ^{c}	13.42 ± 0.37 ^{gh}	1.98 ± 0.08 ^{e}	10 ± 0.41 ^{f}
Red	Control	6.6 ± 0.52 ^{e}	68.3 ± 1.73 ^{ab}	15.77 ± 0.55 ^{bc}	2.99 ± 0.13 ^{b}	14.9 ± 0.52 ^{c}
	30 mM	6.3 ± 0.38 ^{e}	68.6 ± 1.47 ^{ab}	15.64 ± 0.27 ^{bc}	2.69 ± 0.06 ^{d}	13.3 ± 0.68 ^{d}
	60 mM	5 ± 0.33 ^{g}	62.7 ± 2.03 ^{b}	15.22 ± 0.42 ^{cd}	2.34 ± 0.12 ^{de}	8.4 ± 0.53 ^{g}
	90 mM	4 ± 0.31 ^{h}	50.3 ± 1.89 ^{c}	14.31 ± 0.71 ^{ef}	1.87 ± 0.15 ^{ef}	7.2 ± 0.21 ^{gh}
Blue/Red (2:1)	Control	8.1 ± 0.46 ^{c}	70.3 ± 1.45 ^{ab}	16.11 ± 0.34 ^{b}	3.56 ± 0.11 ^{a}	18 ± 0.35 ^{a}
	30 mM	8 ± 0.44 ^{c}	61.8 ± 1.07 ^{b}	15.18 ± 0.27 ^{cd}	2.81 ± 0.14 ^{c}	15.8 ± 0.97 ^{b}
	60 mM	7 ± 0.22 ^{de}	58.3 ± 1.75 ^{bc}	14.81 ± 0.19 ^{de}	2.4 ± 0.13 ^{de}	11.2 ± 0.52 ^{e}
	90 mM	6 ± 0.4 ^{f}	49.4 ± 1.68 ^{c}	13.88 ± 0.29 ^{fg}	1.84 ± 0.1 ^{ef}	9.7 ± 0.68 ^{f}
Blue/Red (1:2)	Control	8.5 ± 0.12 ^{bc}	72.1 ± 1.32 ^{a}	17.23 ± 0.53 ^{a}	3.26 ± 0.17 ^{ab}	17.5 ± 0.32 ^{ab}
	30 mM	8.1 ± 0.54 ^{c}	63.2 ± 3.84 ^{b}	16.09 ± 0.41 ^{b}	2.84 ± 0.21 ^{c}	15.1 ± 0.84 ^{bc}
	60 mM	7.5 ± 0.19 ^{d}	60.1 ± 1.01 ^{b}	14.98 ± 0.36 ^{d}	2.38 ± 0.16 ^{de}	10.2 ± 1.01 ^{f}
	90 mM	7.1 ± 0.25 ^{d}	48.3 ± 1.55 ^{c}	13.54 ± 0.52 ^{fgh}	1.87 ± 0.09 ^{ef}	9.6 ± 0.33 ^{f}
White/Yellow	Control	10.3 ± 0.6 ^{a}	78.4 ± 3.04 ^{a}	16.03 ± 0.53 ^{b}	3.02 ± 0.18 ^{b}	18.3 ± 1.2 ^{a}
	30 mM	9.7 ± 0.38 ^{ab}	70.1 ± 2.53 ^{ab}	16.05 ± 0.28 ^{b}	2.05± 0.12 ^{e}	16.7 ± 1.08 ^{b}
	60 mM	9.2 ± 0.28 ^{b}	63.3 ± 2.58 ^{b}	15.4 ± 0.36 ^{c}	1.84 ± 0.07 ^{ef}	11.9 ± 0.98 ^{e}
	90 mM	8.8 ± 0.31 ^{b}	56.2 ± 1.99 ^{bc}	14.79 ± 0.39 ^{de}	1.35 ± 0.04 ^{f}	9.1 ± 0.45 ^{fg}
Significance	Light (L)	**	**	*	**	*
	Stress (S)	*	*	*	*	*
	L×S	**	**	*	**	**

Values are means ± SE of four replicates. Columns with different letters show significant differences at P ≤ 0.05 (LSD). Significance according to ANOVA, ns, *, **, no significant and significant P ≤ 0.05, 0.01, respectively.

Table 2. Effect of light spectra and salinity stress on leaf gas exchange parameters of marigold.

Light sources	Stress	A (µmol CO_2 m^{-2} s^{-1})	E (mmol H_2O m^{-2} s^{-1})	Gs (mol $H_2Om^2s^{-1}$)	WUEi (µmol $CO_2molH_2O^{-1}$)	SPAD
Blue	Control	20.15 ± 0.69 ^{b}	9.89 ± 0.34 ^{bc}	0.278 ± 0.004 ^{b}	4.9 ± 0.23 ^{a}	43.6 ± 2.2 ^{c}
	30 mM	12.34 ± 0.57 ^{ef}	8.48 ± 0.41 ^{de}	0.181 ± 0.003^{d}	3.5 ± 0.13 ^{b}	48.9 ± 1.97 ^{b}
	60 mM	9.53 ± 0.46 ^{fg}	7.23 ± 0.39 ^{f}	0.136 ± 0.001^{gh}	2.61 ± 0.29 ^{c}	40.6 ± 1.36 ^{d}
	90 mM	6.16 ± 0.55 ^{i}	5.88 ± 0.15 ^{g}	0.096 ± 0.002^{i}	2.01 ± 0.18 ^{d}	29.9 ± 1.15 ^{f}
Red	Control	18.2 ± 0.26 ^{c}	13.18 ± 0.5 ^{a}	0.276 ± 0.006^{b}	2.3 ± 0.26 ^{cd}	48 ± 1.28 ^{ab}
	30 mM	13.6 ± 0.35 ^{e}	7.84 ± 0.44 ^{ef}	0.188 ± 0.008 ^{d}	1.86 ± 0.19 ^{df}	47.8 ± 2.09 ^{b}
	60 mM	9.1 ± 0.29 ^{g}	7.15 ± 0.33 ^{f}	0.141 ± 0.005^{g}	1.65 ± 0.14 ^{f}	41.1 ± 1.17 ^{d}
	90 mM	6.24 ± 0.52 ^{i}	5.57 ± 0.12 ^{gh}	0.098 ± 0.004^{i}	1.26 ± 0.15 ^{g}	30.9 ± 1.94 ^{f}
Blue/Red (2:1)	Control	27.5 ± 0.18 ^{a}	8.76 ± 0.31 ^{cd}	0.362 ± 0.008^{a}	3.41 ± 0.16 ^{b}	51.4 ± 1.82 ^{a}
	30 mM	14.7 ± 0.65 ^{e}	8.21 ± 0.19 ^{def}	0.215 ± 0.007^{c}	2.57 ± 0.11 ^{c}	49.7 ± 1.08 ^{ab}
	60 mM	12.2 ± 0.01 ^{f}	7.30 ± 0.30 ^{f}	0.158 ± 0.005^{f}	1.98 ± 0.09 ^{d}	40.4 ± 2.01 ^{d}
	90 mM	8.32 ± 0.14 ^{h}	4.85 ± 0.27 ^{hi}	0.112 ± 0.003^{hi}	1.56 ± 0.07 ^{fg}	33 ± 1.34 ^{f}
Blue/Red (1:2)	Control	25.61 ± 0.72 ^{a}	8.90 ± 0.55 ^{cd}	0.357 ± 0.005^{a}	3.17 ± 0.18 ^{b}	49.1 ± 2.16 ^{ab}
	30 mM	14.3 ± 0.29 ^{e}	8.13 ± 0.32 ^{def}	0.224 ± 0.002^{c}	2.42 ± 0.11 ^{c}	49.5 ± 1.24 ^{ab}
	60 mM	12.2 ± 0.37 ^{f}	7.28 ± 0.4^{f}	0.164 ± 0.006^{ef}	1.75 ± 0.17 ^{f}	39.9 ± 1.71 ^{de}
	90 mM	8.08 ± 0.11 ^{h}	4.60± 0.15 ^{hi}	0.114 ± 0.001^{h}	1.32 ± 0.08 ^{fg}	32.1 ± 2.12 ^{f}
White/Yellow	Control	16.8 ± 0.51 ^{d}	9.9 ± 0.35 ^{b}	0.238 ± 0.006^{c}	3.11 ± 0.21 ^{bc}	48 ± 1.68 ^{b}
	30 mM	10.23 ± 0.42 ^{f}	7.4 ± 0.22 ^{f}	0.178 ± 0.004^{de}	1.72 ± 0.13 ^{f}	48 ± 1.68 ^{b}
	60 mM	8.47 ± 0.33 ^{h}	7.48 ± 0.13 ^{f}	0.118 ± 0.002^{h}	1.52 ± 0.09 ^{fg}	39.8 ± 1.15 ^{de}
	90 mM	4.94 ± 0.19 ^{j}	4.63 ± 0.41 ^{hi}	0.084 ± 0.003^{j}	1.18 ± 0.12 ^{g}	33.6 ± 1.18 ^{f}
Significance	Light (L)	**	**	*	**	**
	Stress (S)	*	**	*	**	**
	L×S	*	**	**	**	**

Values are means ± SE of four replicates. Means with different letters in each columns show significant differences at P ≤ 0.05 (LSD). Significance according to ANOVA, ns, *, **, no significant and significant P ≤ 0.05, 0.01, respectively.

K at all salinity levels (Fig 4c). Under salt stress, plants treated with blue/red 1:12 ratio light had the highest concentration of K in shoot and root (Fig 4c, d).

Total phenol and flavonoid content: According to

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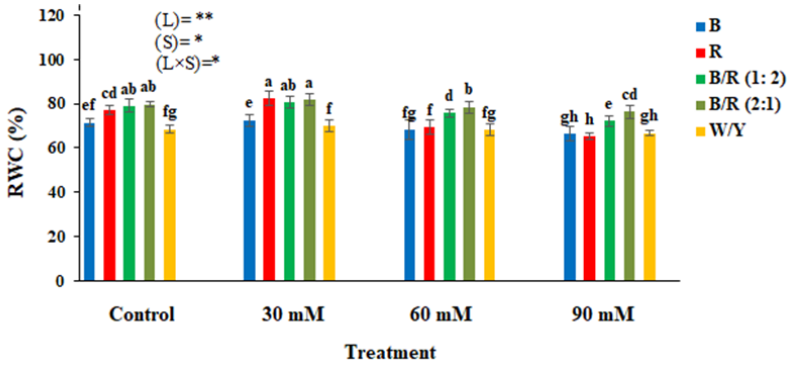

Figure 2. Changes in RWC index under the effect of five light spectrum levels and four salt stress levels. Means, followed by the same letter, are not significantly different according to the LSD (P≤0.05). Vertical bars indicate the standard errors of four replicates. SAS software version 9.4 was used for data analysis.

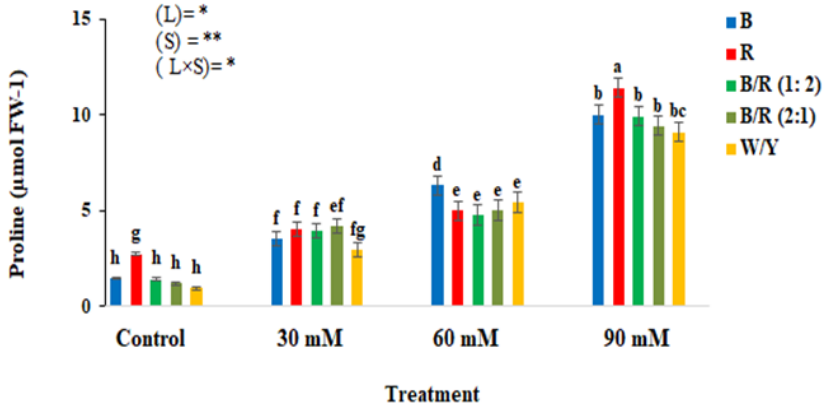

Figure 3. Changes in proline under the effect of five light spectrum levels and four stress levels. Means, followed by the same letter, are not significantly different according to the LSD (P≤0.05). Vertical bars indicate the standard errors of four replicates. SAS software version 9.4 was used for data analysis.

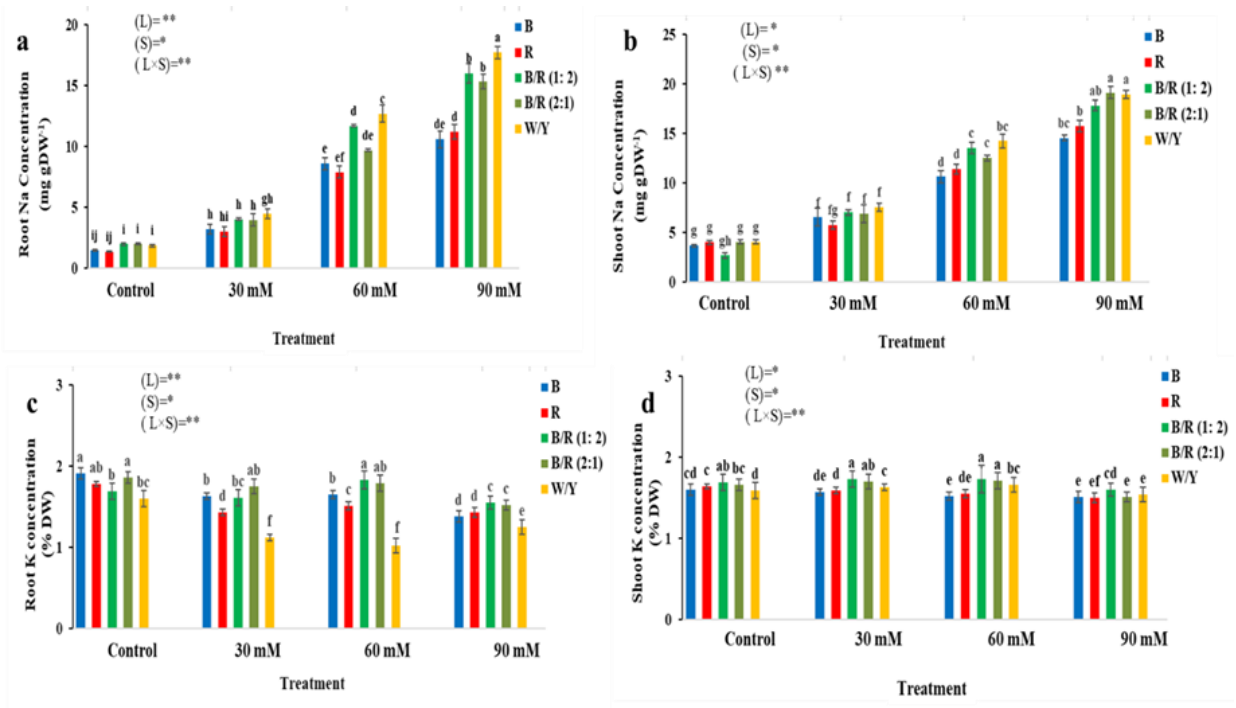

Figure 4. Interaction of five levels of light spectrum and four levels of salinity stress treatment on nutrient element concentration. Values are means± SE of three replicates. Bars with different letters show significant differences at P≤0.05 (LSD). Significance according to ANOVA, ns, *, **, no significant and significant P≤0.05, 0.01, respectively.

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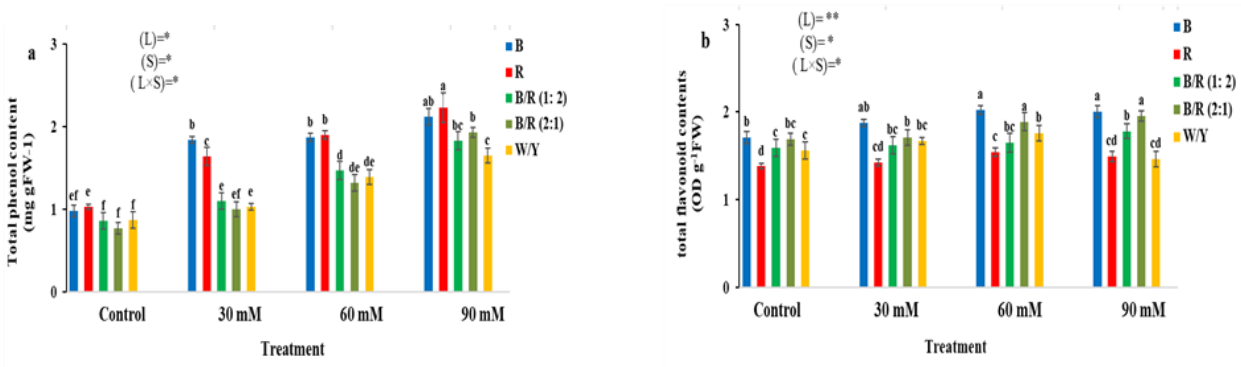

Figure 5. Interaction of five levels of light spectrum and four levels of salinity stress treatment on total phenol (a) and flavonoid content (b). Values are means±SE of three replicates. Bars with different letters show significant differences at P≤0.05 (LSD). Significance according to ANOVA, ns, *, **, no significant and significant P≤0.05, 0.01, respectively.

the results, stress and light spectrum had a significant effect on total phenol and flavonoid content (Fig 5a, b). Salinity stress reduced the total phenol compared to non-stress conditions. The maximum phenol content was observed in red light and 90 mM salinity stress. The lowest level of this parameter was observed in the control and Blue/Red (2:1) treatments. In the control treatment and at all salinity levels, blue light accounted for the highest amount of flavonoids (Fig 5 b).

Discussion

Marigold (*Calendula officinalis* L.) is a well-known medicinal plant, widely used for the symptomatic treatment of minor inflammations of the skin (Hao *et al*., 2022). Similarly, to other pharmaceutical plant species, the growth, yield, and chemical compositions of these species are affected by environmental factors when grown under open-field conditions. However, with the recent fast development of LED grow lighting systems and the increasing efficacy of these systems, growing pharmaceutical plants, such as marigold, vertically under controlled environmental conditions is now viable and has potential commercial value (Mohammadi *et al*., 2020). In the plant factory system, LEDs are used as the sole source of lighting and provide a unique tool for promoting growth, yield, and quality. However, plant species respond differently to lighting conditions, and therefore it is crucial to vary light spectra and intensity to suit the requirements of individual plant species (Rihan *et al*., 2020).

Numerous studies have shown that stresses inhibit photosynthesis in different plant species (Liu and Shi, 2010). The first response of the plant to salinity stress is leaf area limitation and low growth (Yadav *et al*., 2019). Salt stress leads to leaf chlorosis, a decrease in leaf area, and reduced photosynthesis due to chlorophyll degradation (Zahra *et al*., 2022). A combination of blue and red light spectra is crucial for leaf area and plant biomass production (Johkan *et al*., 2010). Results show that under the influence of salinity, there is a decrease in the RWC. The accumulation of salt in the root zone prevents roots from absorbing water by reducing the osmotic potential, thus decreasing the water content of the leaf (Sharma *et al*., 2019) and under salt stress, plants accumulate inorganic ions in the vacuole to lower the water potential of the cell.

Plants can receive and sense changes in light quality through light receptors and regulate their growth and development through signaling pathways. It is well established that the morphological, physiological and nutritional quality of plants is affected by the quality and intensity of light (Shafiq *et al*., 2020). Chloroplasts absorb and use mainly blue and red light for photosynthesis (Li *et al*., 2020). Our study investigated the effect of some light spectra as supplementary light on the vegetative and reproductive processes of marigold. Some light spectra have been shown to help plants become more resistant to biological and abiotic stresses (Malekzadeh Shamsabad *et al*., 2022). Our results showed that a combination of blue and red light, especially with a 2:1 ratio, had a stronger effect on vegetative traits and could reduce stress more than other light spectra. Blue light is essential for chlorophyll biosynthesis. Studies suggest that a combination of blue and red wavelengths of LED lighting is usually chosen to enhance the efficiency of plant photosynthesis (Soufi *et al*., 2023). In addition, several studies have shown that the combination of blue and red spectrum plays an important role in leaf area and plant biomass (Roosta *et al*., 2024). Fang *et al*. reported that shoot dry weight increased under blue/red light (Fang *et al*., 2021). This increase was attributed to the effect of blue/red light on leaf number and leaf area. Our results showed that under stress conditions, blue/red light caused a significant increase in leaf area compared to other light spectra. Experiments show that blue and red LED light increases vegetative and reproductive traits of plants under greenhouse conditions. Our results also showed that blue/red light under salt stress caused the highest increase in leaf area. These results can show the importance of using these light spectra on plant growth under stress conditions.

According to our studies, changes in light spectra significantly affect the photosynthetic system of marigold plants. We also found that under salinity stress, the combination of red and blue light had a positive effect on net photosynthesis rate. Under stress conditions, plants grown under blue light had the

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highest SPAD index. In our other experimental results, it was shown that under stress conditions, the spectra of blue and red light and their combination affected the gas exchange parameters of plants. These spectra increased the CO_2 uptake rate (A) and WUEi. It can be concluded that blue and red light improves the resilience of plants to stress conditions by affecting the photosynthetic efficiency of the plant. Previous studies have shown that a lack of blue light inhibits chlorophyll biosynthesis in pink hybrids (Terfa *et al*., 2013).

Salinity stress decreased water use efficiency (WUEi) compared to the control, blue/red light had a significant positive effect on this parameter and maintained it at the control level under salinity. WUEi under salt stress was also improved by red and blue light used separately. These results revealed that growing plants under a combination of light spectra enabled the plants to develop a photosynthesis apparatus with lower vulnerability than the photosynthesis apparatus in monochromatically grown plants in response to salinity stress.

Flavonoid belong to phenolic compounds and valuable antioxidant compounds that increase the tolerance of plants to salt stress (Shomali *et al*., 2022). According to our results, blue light could increase total flavonoid content under salinity stress conditions. Blue light is one of the effective factors to increase flavonoid biosynthesis.

Na ions are the major toxic ions in saline soils. Low Na and high K levels in the cytoplasm are essential for maintaining the activity of some enzymes (Zhu, 2003). Plants subjected to salinity stress inevitably accumulate large amounts of sodium. Increasing sodium prevents the plant from absorbing potassium and decreasing the potassium content in the plant (Hasegawa *et al*., 2000). One of the most important roles of K is water balance and solute transport in the woody vessels of plants. Potassium plays an important role in solute transport in the xylem and water balance of plants (Koch *et al*., 2019). Plants exposed to salinity stress take up large amounts of sodium, which prevents the uptake of K and reduces the K content in the plant (Hasegawa *et al*., 2000). Our results showed that blue/red light under stress increased potassium content compared to the control. Sun *et al*. reported that plant uptake and accumulation of K under salt stress increased plant resistance (Sun *et al.*, 2015). The transport of K, Ca, and Mg is disrupted by Na under salinity conditions and may interfere with plant metabolism and reduce plant growth (Koch *et al*., 2019).

Conclusions

Analysis of the morphological, physiological, and elemental characteristics of marigold plants indicates that plants employ varied strategies against abiotic stress depending on light quality. The findings reveal that the combination of blue and red light spectra influences the absorption of elements and the photosynthetic apparatus of plants, thereby enhancing both vegetative and reproductive growth and bolstering plant resistance to stress. While white/yellow light enhances vegetative traits of marigold plants under non-stress conditions, exposure to additional blue, red, and particularly the combination of blue and red light enhances stress tolerance under adverse conditions. The spectra of light impact plant resistance to stress by influencing element absorption and photosynthetic performance. Understanding these effects under diverse growing conditions lays the groundwork for manipulating light spectra to bolster plant resistance to stress. Although LED technology holds promise for greenhouse plants, further research is warranted to investigate its effects on various plants and under differing conditions. Incidence spectrum and photon flux density emerge as pivotal factors in determining the appropriate light for a plant, dictating plant growth in response to light conditions. In conclusion, light quality can exert a profound influence on numerous aspects of marigold plant morphology and physiology, emphasizing the importance of a specific quantity and quality of light for optimal species or cultivar growth.

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