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LED Lights and Glycine Promote Biomass, Leaf Color Changes, and Secondary Metabolites Accumulation in *Hyoscyamus niger* L.

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ABSTRACT

Light-emitting diode (LED) lighting holds significant promise in agriculture, particularly for the production of secondary metabolites (SM). With features like high energy efficiency, long lifespan, and flexibility in application, LEDs are expected to outperform traditional lighting systems in enhancing SM production in the future. This study investigated the effects of LED lighting and glycine treatments on the production of tropane alkaloids in *Hyoscyamus niger* L. Starting at the transplant stage, plants were subjected to four light treatments (white, blue, red LEDs, and the control in greenhouse conditions) while glycine foliar spray was applied at three concentrations (0, 40, and 80 mg L^{-1}) at 10-day intervals. Fourteen days after the final treatment, yield efficiency, tropane alkaloids, phenols, and flavonoids were evaluated. The highest total carbohydrate content was observed under red LED light combined with 80 mg L^{-1} of glycine. High-performance liquid chromatography (HPLC) assays revealed that both LED light and glycine positively influenced the accumulation of two key metabolites, scopolamine and atropine. The highest scopolamine concentration (22.61 mg g^{-1}) and atropine levels were recorded under blue LED light combined with glycine at 40 and 80 mg L^{-1}.

Introduction

Henbane (*Hyoscyamus niger* L.), a member of the Solanaceae family, is native to Southwest Asia, particularly Iran, and North Africa (Raghvan et al., 1996). This genus is renowned as a primary source of tropane alkaloids, including scopolamine, hyoscyamine, and atropine. Due to their complex chemical structures, synthetic production of these alkaloids is expensive, which is why they are typically extracted from Solanaceae plants. Alkaloid production generally begins in the roots and is then transported to the shoots (Stewart, 2013).

Plant secondary metabolites (SMs) are compounds that, while not involved in basic life processes, play crucial roles in a plant's interaction with its environment (Namdeo, 2007; Khalighi et al., 2021). These metabolites often function in attracting pollinators or in defense mechanisms against predators, as well as responding to both biotic and abiotic stressors. Unlike primary metabolites, the distribution of SMs in plants is limited, with many compounds found only in a few species or specific genera. The production of SMs is generally low (less than 1% dry weight) and depends on the plant species, its physiological state, and growth stage (Namdeo, 2007; Raghavan et al., 1996).

Plants exhibit a variety of physiological and morphological responses to microbial, chemical, and physical factors known as elicitors. Elicitation is a process that induces or enhances the synthesis of SMs, allowing plants to better

survive, resist environmental pressures, and compete (Guo et al., 2022). Numerous studies have explored how various environmental stressors can increase bioactive compound production in plants (Hasan et al., 2017).

Light plays a vital role in plant photosynthesis and growth, significantly influencing the accumulation and quality of secondary metabolites. For example, sunlight promotes the accumulation of coumarin in guava plants. A shorter photoperiod reduces coumarin levels in leaves and stems, while a longer photoperiod significantly increases coumarin content. Therefore, light intensity and photoperiod are crucial factors that enhance yield and improve photosynthetic efficiency (Naik et al., 2016).

Amino acids play a key role in stimulating the natural growth of plants and are increasingly used to enhance the quality of plant products (Khan et al., 2019; Giglou et al., 2023). Additionally, amino acids have been shown to impact the production of SMs. Foliar application of amino acids, such as glycine, can act as a growth stimulator, influencing both plant growth and quality (Noroozlo et al., 2019).

Glycine, or 2-aminoethanoic acid (NH_2CH_2COOH), is the simplest amino acid found in organisms and is the smallest of the 20 amino acids that make up proteins. It plays an important physiological role in plants by activating photosynthesis (Ciuzan et al., 2015). Due to its small molecular weight and role in cell membrane permeability, glycine is easily absorbed by plants, often faster than other forms of nitrogen, likely due to specialized transporters that facilitate amino acid uptake into plant organs. Studies have shown that amino acids, including glycine, can enhance the production of metabolites (Khattab et al., 2016).

LED lighting, on the other hand, provides a cost-effective light source for plant growth, with advantages such as long lifespan, small size, monochromatic light, and adjustable intensity (Irshad et al., 2018). As artificial light sources in controlled plant growth environments, LEDs offer high energy conversion efficiency, adjustable light intensity and quality, specific wavelengths, and low heat output. Research on the effects of LED lighting on different plant species has shown that various colors of LEDs influence both morphological and physiological characteristics (Lian et al., 2019).

However, few studies have examined the combined effect of LED lighting and amino acids on the production of secondary metabolites. Therefore, this study aimed to evaluate the impact of different concentrations of glycine and four LED light colors (blue, red, white, and natural greenhouse light) on *Hyoscyamus niger* L. The concentration of atropine and scopolamine was analyzed using HPLC, while total phenol content (TPC), total flavonoid content (TFC), and total antioxidant content (TAC) were also measured. This study provides a potential theoretical basis for understanding how LED light and glycine influence SM biosynthesis in *H. niger* L.

Materials and Methods

Plant materials and experimental design

Henbane seeds (*Hyoscyamus niger* L.) were obtained from the National Plant Gene Bank of Iran (Accession no. TN-82-763). After washing, the seeds were soaked in water for 24 h, then stored in a wet sand bed at 5°C for 28 days. Once the seeds were conditioned, they were planted in plug trays and maintained in a greenhouse until germination, with seedlings grown to the 3-4 leaf stage. At the 4-leaf stage, healthy seedlings were transplanted into pots measuring 20 x 40 cm in diameter, with a volume of 5 L.

Following the method outlined by Giglou et al. (2023), the pots were transferred to light boxes (100 x 100 cm) for exposure to four different light treatments: white (W), blue (B), red (R) LEDs, and normal greenhouse conditions as the control (C). The LED lamps used were 1200 mm × 24 mm in size with a nominal power of 16 W. Each lamp comprised 96 sets of LEDs, with an equal distribution of blue, red, and white lights. The lamps operated at a nominal voltage of AC 85-220 V, with an operating current of 100 mA and a frequency of 60.50 Hz. The peak emission wavelengths for the red and blue lights were 620-630 nm and 460-470 nm, respectively.

Fourteen days after the light treatments began, foliar applications of glycine (CAS No: 56-40-6) at concentrations of 0, 40, and 80 mg L^{-1} were applied three times at weekly intervals. The experiment was designed as a factorial arrangement in a completely randomized design (CRD) with three replications. The pots were arranged in four separate light boxes: three boxes for white, blue, and red LED treatments, and one box in the greenhouse without LED light serving as the control (C). In each box, 30 pots were divided into three groups of 10 pots, with each group representing a replication. The glycine treatments were applied according to the experimental design, with 4 light treatments, 3 glycine treatments, and 3 replications per treatment, for a total of 120 pots. Fourteen days after the final glycine application, the following traits were measured for both aerial parts and roots: plant height, fresh matter (FM), and dry matter (DM).

Chlorophyll fluorescence (Chlf) and color measurements

Chlorophyll fluorescence was measured using fully developed leaves randomly selected from each plant, utilizing a chlorophyll fluorimeter (Hansatech Instruments Ltd., King's Lynn, United Kingdom). Prior to measurement, the clamps of the device were adapted to darkness for approximately 20 min. Once adapted, the diode was connected to the clamp, the clamp valve was opened, and the data were recorded (Maxwell and Johansson, 2000; Seifikalhor et al., 2020; Seif et al., 2021). Leaf color in *Hyoscyamus niger* L. was measured using a colorimeter device (CR-10 plus, Konica Minolta Inc., Tokyo, Japan) (Table 1).

Table 1. Terms and formulae used in the analysis of fast chlorophyll fluorescence (Giglou et al., 2022).

Term and formulae	Definition
F_o	Fluorescence is emitted when all reaction centers (RCs) are open
F_m	Maximum fluorescence emitted when all RCs are closed
$F_v = F_m - F_o$	Maximum variable fluorescence
$F_v/F_m = 1-(F_o/F_m)$	The maximum quantum yield of primary photochemistry

Total carbohydrates (TC)

The TC content was determined using the phenol-sulfuric acid method. Briefly, 0.1 g of dry plant tissue was mixed with 10 mL of 70% ethanol and stored at 4°C for one week. During this period, the samples were stirred daily to facilitate the extraction of dissolved carbohydrates. After one week, 1 mL of the supernatant was collected and diluted to 2 mL with distilled water. To this solution, 1 mL of 5% phenol and 5 mL of 48% sulfuric acid were added, followed by thorough mixing using a vortex.

The mixture was then heated in a water bath at 65°C for 20 min, after which it was cooled to room temperature to stop the reaction. The absorbance of the samples was measured using a spectrophotometer at a wavelength of 485 nm. A standard curve was prepared using glucose solutions with concentrations ranging from 0 to 10 mg 100 mL^{-1}. Based on the dry weight of the samples, the TC content was calculated and expressed as mg g^{-1} of dry matter (DM) (Irigoyen et al., 1992).

Preparation of dry matter for measuring total phenol content (TPC), total flavonoids content (TFC), and total antioxidant capacity (TAC)

Fruits were dried in an oven at 40°C for 96 h, after which the dried material was milled. For extraction, 1 g of each sample was soaked in 50 mL of 80% methanol for 48 h at room temperature. After the extraction period, the extracts were filtered using Whatman No. 4 filter paper. The solvent was then evaporated at a temperature below 55°C. All subsequent experiments were conducted at 4°C (Pourmorad, 2006).

Total phenol content (TPC)

The TPC content was determined using a method described by Meda et al. (2004), with gallic acid as the standard for generating a standard curve. The TPC was expressed as mg of gallic acid equivalent g^{-1} DW (mg GAE g^{-1} DW).

Total flavonoids content (TFC)

To measure the total flavonoid content, 500 µL of each extract was mixed with 1.5 mL of 80% methanol, 100 µL of 10% AlCl2 solution, 100 µL of 1 M sodium acetate solution, and 2.8 mL of distilled water. The absorbance of the mixture was measured at 415 nm after 40 min, with a blank containing all reagents except the extract (which was replaced by 80% methanol). Quercetin was used to create the standard curve, and the results were reported as mg of quercetin equivalent g^{-1} DW (mg QE g^{-1} DW) (Mita et al., 1997).

Total antioxidant capacity (TAC)

The total antioxidant capacity was assessed using the DPPH method as described by Miliauskas et al. (2004). Different concentrations of the extract were prepared to achieve final mass ratios of extract to DPPH at approximately 3:1, 1.5:1, and 0.75:1. The extract was mixed with 2 mL of a 0.004% methanolic DPPH solution, and a control solution was prepared with 2 mL of DPPH and 2 mL of methanol. The solutions were incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm against the methanol control. The percentage of free radicals (I%) in each extract was calculated using the following formula:

$$I\% = (A\ control - A\ sample) / A\ control \times 100$$

Extraction of tropane alkaloids (TA), quercetin, and gallic acid

TA were extracted from the plant material following drying at 45°C, according to the method described by Deveci et al. (2022). After drying, the plant material was ground into a fine powder using a pestle and mortar. The extraction from *Hyoscyamus niger* was conducted using the protocol outlined by Jakabova et al. (2012).

Measurement of quercetin and gallic acid using the spectrophotometric method

Solutions of quercetin and gallic acid were prepared at concentrations of 0.25, 0.5, 1, and 2 mg in 100 mL volumetric flasks. The absorption spectra for both compounds were determined using a spectrophotometer (ND One, Thermo Fisher, USA), and their maximum absorption values were recorded. Linear regression analysis was performed to generate calibration curves for both quercetin and gallic acid, using the concentration of the standard solutions and their corresponding absorbance values. The maximum absorption for quercetin was found to occur at a wavelength of 405 nm, while for gallic acid, it was recorded at 310 nm (Table 2).

Table 2. Linear regression equation and correlation coefficient for gallic acid, quercetin, scopolamine and atropine (n = 4).

Compounds	Linear regression equation	Correlation coefficient
Gallic Acid	y = 8.2487x + 3.7725	0.9835
Quercetin	y = 6.1515x + 3.0692	0.9752
Scopolamine HBr.3H2o	y = 4404x + 1129.2	0.9417
Atropine	y = 7605.5x + 856.04	0.9634

Determination of tropane alkaloids by HPLC

The HPLC analysis of tropane alkaloids was conducted at the Central Laboratory of the University of Mohaghegh Ardabili using a Well Chrome 2000 system (Knauer, Germany) equipped with a Eurospher C18 column (250 × 4.6 mm) and a UV detector set at a wavelength of 220 nm. An isocratic elution method was employed using a mixture of water and acetonitrile (60:40) at a flow rate of 0.5 mL min-1, ensuring optimal separation and sensitivity for the tropane alkaloids.

Statistical analysis

The experimental data were analyzed as a factorial experiment based on a completely randomized design (CRD) using ANOVA and Duncan's multiple range test ($P<0.05$). The analyses were performed using SAS 9.1 for Windows (SAS Institute Inc., Carolina, USA) and SPSS21 for Windows (IBM SPSS Statistics, New York, USA) ($P<0.05$).

Results

Yield

Over the 6-week period from transplanting to pots until sampling, the application of different LED lights resulted in significant variations in plant height. Our findings revealed that the greatest height changes occurred in plants exposed to BLED (Table 3), with the highest plant height (49.2 cm) observed in those treated with 80 mg L^{-1} glycine under BLED conditions (Table 3). While the plants in the BLED box progressed to the flowering stage, those in the other LED boxes remained in the rosette stage, only showing an increase in leaf number.

In treatments with different glycine concentrations and lighting conditions, it was evident that the application of glycine enhanced plant height across all LED boxes, as well as under normal greenhouse conditions. For fresh matter (FM) and dry matter (DM) of aerial parts and roots (FMR), the data indicated a direct correlation between FM, DM, and plant height. Moreover, the combined use of glycine and different LED lights significantly increased the biomass of H. niger L.

The highest FM (19.32 g for aerial parts and 1.66 g for roots) and DM (3.07 g for aerial parts and 0.82 g for roots) were recorded in plants exposed to BLED. Additionally, foliar application of 80 mg L^{-1} glycine under BLED conditions had the most pronounced effect on both FM and DM (Table 3).

Chlorophyll fluorescence (ChlF) and leaf color changes

ChlF parameters were used to assess the efficiency of photosystem II activity (Table 4). The highest F_0 value (315.59) was observed in plants grown under WLED conditions, while the lowest F_0 value was recorded in plants grown under normal light conditions. In *H. niger*, the highest values of Fm (1520.3) and Fv (1252) were noted in plants exposed to RLED. However, there were no significant differences between the Fm values of plants treated with RLED and BLED light. These results suggest that plants grown under RLED and BLED conditions experienced higher levels of light stress compared to those under normal light conditions (Table 4).

Table 3. Interaction of LED light and glycine on yield efficiency in *H. niger* L. plants.

LED light	Glycine (mg L^{-1})	plant height (cm)	Plant FM (g)	Plant DM (g)	root FM (g)	root DM (g)
C	0	3.33 ± 1.001	5.59 ± 0.79	1.2 ± 0.32	0.703 ± 0.16	0.356 ± 0.03
	40	3.66 ± 0.55	5.62 ± 0.92	1.3 ± 0.42	0.88 ± 0.14	0.416 ± 0.031
	80	4.36 ± 0.666	6 ± 0.86	1.46 ± 0.39	1.04 ± 0.06	0.56 ± 0.07
R	0	3.43 ± 0.91	5.56 ± 0.39	1.36 ± 0.19	0.88 ± 0.1	0.376 ± 0.04
	40	4.43 ± 0.321	6.78 ± 0.21	1.58 ± 0.17	1.13 ± 0.13	0.596 ± 0.09
	80	4.86 ± 0.305	7.04 ± 0.738	1.66 ± 0.19	1.25 ± 0.07	0.713 ± 0.025
B	0	35 ± 1.67	13.4 ± 0.55	2.13 ± 0.1	1.14 ± 0.08	0.56 ± 0.05
	40	41.51 ± 3.14	17.75 ± 0.91	2.66 ± 0.27	1.51 ± 0.14	0.736 ± 0.055
	80	49.2 ± 2.29	19.32 ± 0.49	3.07 ± 0.13	1.66 ± 0.08	0.82 ± 0.065
W	0	3.36 ± 0.49	4.78 ± 0.86	0.97 ± 0.24	0.77 ± 0.09	0.386 ± 0.075
	40	4.53 ± 0.32	5.17 ± 0.33	1.09 ± 0.12	0.95 ± 0.1	0.533 ± 0.07
	80	3.73 ± 0.404	5.78 ± 0.29	1.52 ± 0.21	1.02 ± 0.16	0.553 ± 0.1

LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. ± indicate standard deviation.

Table 4. Effect of LED light on chlorophyll fluorescence in *H. niger* L.

LED light	F_0	Fm	Fv
	Chlorophyll fluorescence		
C	215.78^{c}	1233.89^{b}	1018^{b}
R	268.3^{b}	1520.3^{a}	1252^{a}
B	255.93^{b}	1418.78^{a}	1162^{ab}
W	315.59^{a}	1054.41^{c}	738.81^{c}

LED, C, B, R, and W refer to light color, normal Light conditions, blue, red, and white, respectively. The same letter within each column indicates no significant difference among treatments using Duncan's Multiple Range Test.

The color of the leaves underwent noticeable changes after six weeks of exposure to different LED lights and glycine treatments. The results indicated that the L* values of *H. niger* L. significantly increased under RLED, BLED, and control (C) conditions in the presence of glycine, compared to plants grown under WLED (Fig. 1). Conversely, glycine treatment in WLED conditions led to a decrease in the L* index, as shown in Figure 1. In terms of the a* index, the most significant changes (-11.680°) were observed in plants exposed to BLED, where the leaves appeared yellow. The lowest a* value (-14.83°) was detected in plants grown under RLED (Table 5).

TC content

Our results indicated that the application of LED light combined with foliar application of glycine significantly increased the total carbohydrate content in *H. niger* L. In plants exposed to red (RLED) and white (WLED) LED lights, the application of a medium concentration of glycine (40 mg L^{-1}) notably enhanced the total carbohydrate content (Fig. 2). Similarly, in plants grown under blue LED (BLED) and control (C) conditions, the TC content increased with higher glycine concentrations. The highest TC level (0.511 mg g^{-1}) was recorded in both light conditions when glycine was applied at 80 mg L^{-1}. Furthermore, the TC content in plants under BLED with 80 mg L^{-1} glycine exhibited a significant increase compared to those in RLED, WLED, and C conditions (Fig. 2).

Total phenol (TPC) and flavonoids (TFC) content

The combination of LED light and glycine application significantly enhanced the accumulation of total phenolic content (TPC) and total flavonoid content (TFC) in *H. niger* L. Glycine, at both concentrations used, resulted in increased levels of TPC and TFC. The analyses revealed that the lowest TPC (50.61 mg g^{-1}) and TFC (20.76 mg g^{-1}) were observed in plants grown under control (C) conditions without glycine (Figs. 3 and 4). The use of LED lights notably increased the TFC in the studied plants compared to the normal light conditions (C). The highest TFC was recorded in plants grown under blue LED (BLED) with 80 mg L^{-1} of glycine (Fig. 4). Conversely, the highest TPC (72 mg g^{-1}) was observed in plants under BLED with 40 mg L^{-1} of glycine (Fig. 3).

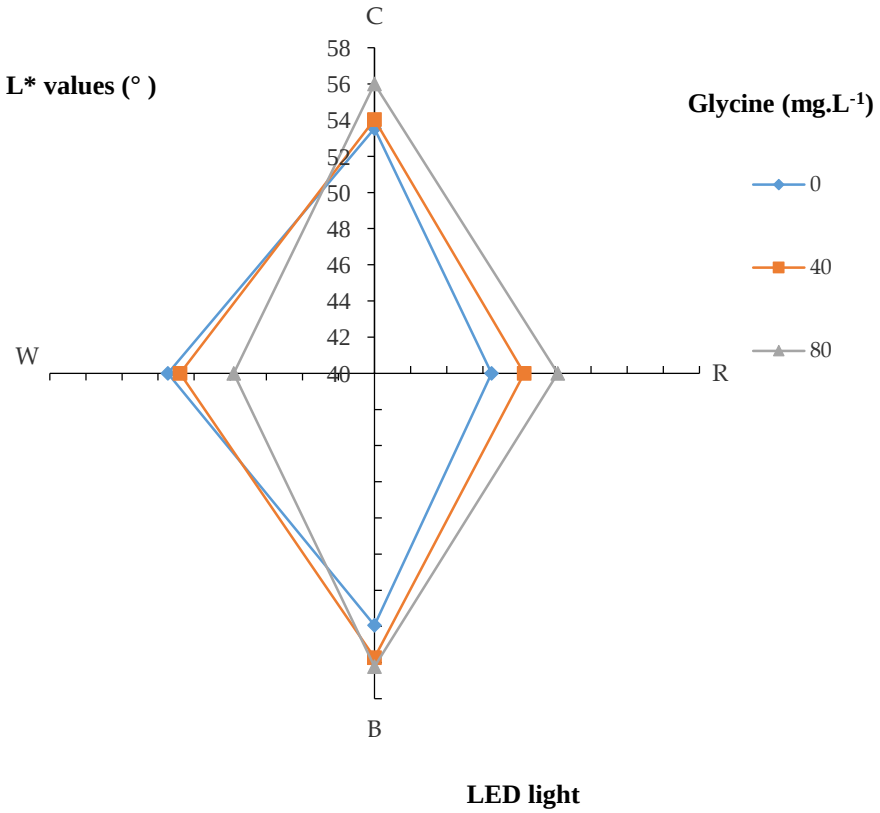

Fig. 1. Effect of LED light and glycine on L* values (lightness). LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Glycine at 0, 40 and 80 mg L^{-1}.

Table 5. Effect of LED light and glycine on leaf color. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively.

		a* (°)	b* (°)
LED light	C	-14.64^{b}	23.059^{ab}
	R	-14.83^{b}	18.392^{b}
	B	-11.498^{a}	25.452^{a}
	W	-13.34^{ab}	21.27^{ab}
Glycine (mg L^{-1})	0	-10.40^{a}	23.504^{a}
	40	-13.424^{b}	20.83^{ab}
	80	-13.669^{b}	19.223^{b}

The “a” value showed the red-green component of the “a” color (-128 to 128), where positive “a” and negative values of “a” indicate red and green values, respectively. While parameter “b” (-128 to 128) tends to be yellow and tend to be blue. The same letter within each column indicates no significant difference among treatments using Duncan’s Multiple Range Test.

Total antioxidant capacity (TAC)

Total antioxidant capacity is one of the strongest indicators reflecting the impact of LED light and glycine in this experiment. The DPPH method was employed to measure total antioxidant capacity. The results indicated that the application of 40

and 80 mg L^{-1} of glycine under the various colored LED conditions and normal light significantly increased total antioxidant capacity in the plants. The highest enhancement in total antioxidant capacity (33.46%) was observed in plants exposed to blue LED (BLED) with 80 mg L^{-1} of glycine (Fig. 5).

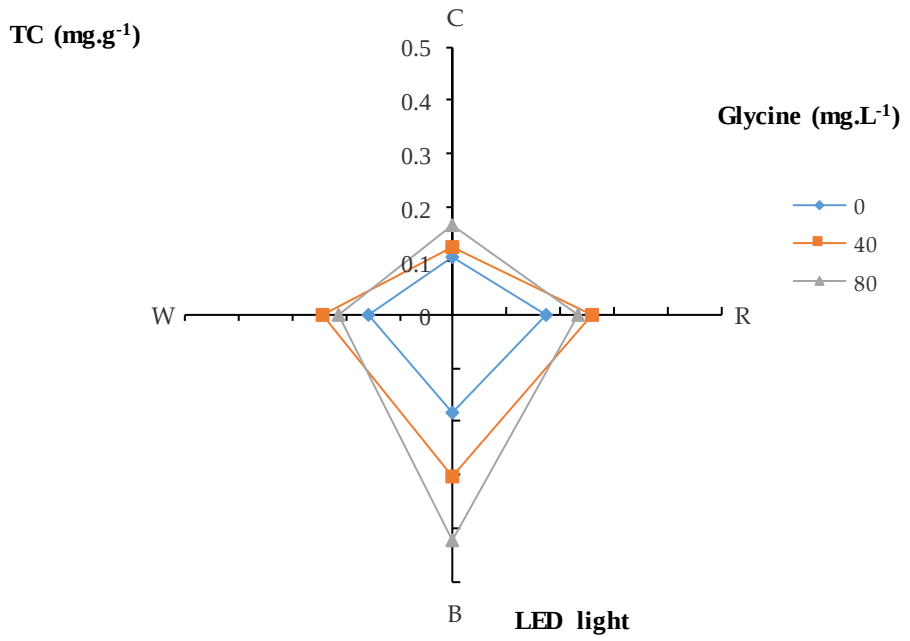

Fig. 2. Interaction of LED light and glycine on TC in *H. niger* L. plants. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively, glycine at 0, 40 and 80 mg L^{-1}.

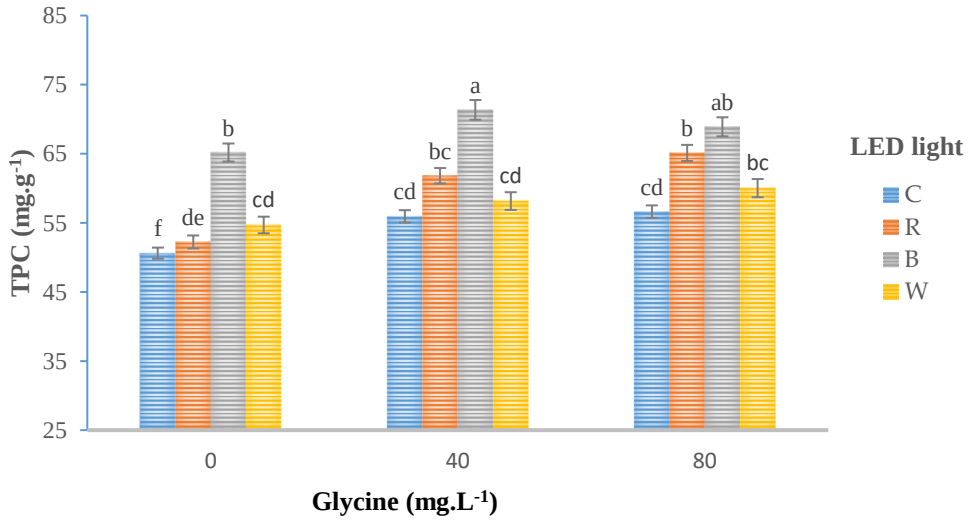

Fig. 3. Effects of glycine on TPC in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ($P<0.05$).

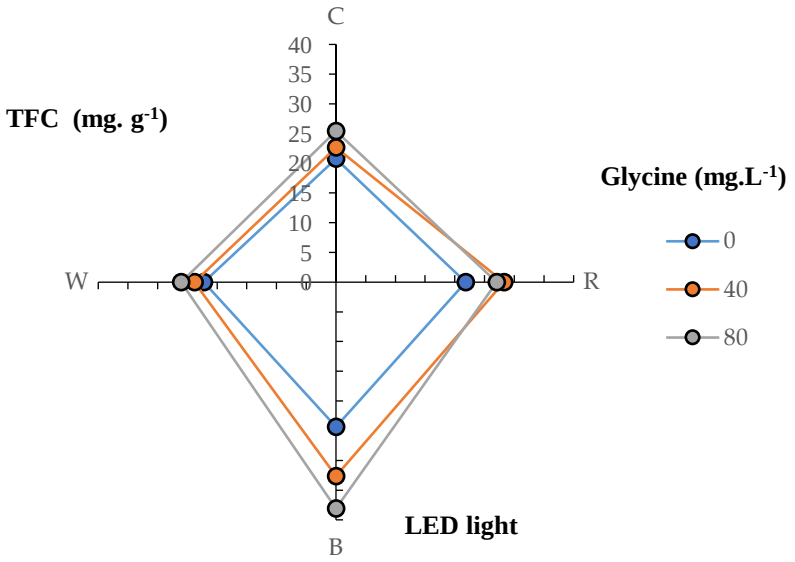

Fig. 4. Effects of glycine on TFC in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively, glycine at 0, 40, and 80 mg L^{-1}.

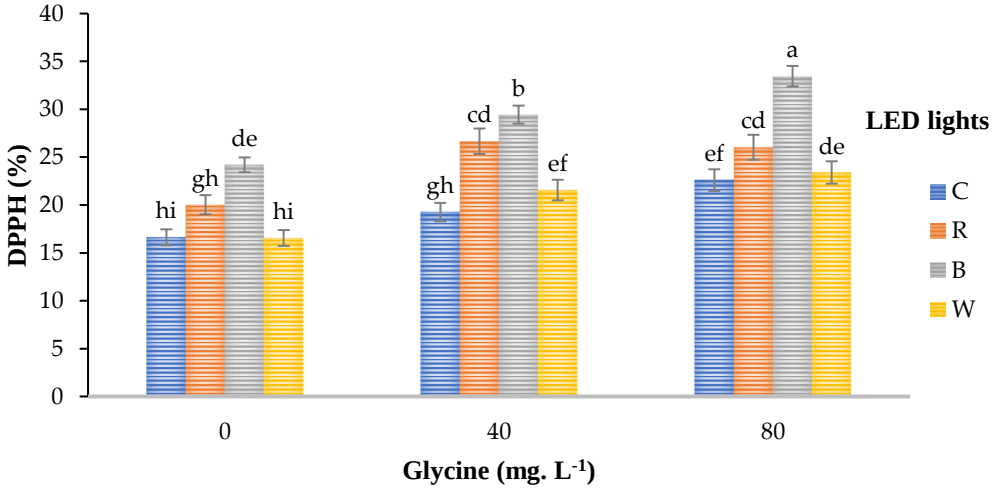

Fig. 5. Effects of glycine on TAC in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ($P<0.05$).

Gallic acid (GA) and quercetin.

The results of this study demonstrated that increasing glycine concentrations under colored LED light conditions had a more significant impact on enhancing GA and quercetin levels in *H. niger* plants compared to normal light conditions. Specifically, the highest amounts of GA and quercetin were obtained at 80 mg L^{-1} of glycine (Fig. 6). Notably, the highest GA concentration (3.24 mg g^{-1}) and quercetin content were recorded in plants grown under blue LED (BLED) light at 80 mg L^{-1} of glycine (Figs. 6 and 7).

Tropane alkaloids (TA)

To assess the effects of LED lights and glycine on tropane alkaloids, high-performance liquid chromatography (HPLC) was utilized to measure atropine and scopolamine levels. The lowest concentration of atropine in *H. niger* was recorded at 2.57 mg g^{-1} in plants grown under WLED light. In contrast, atropine levels significantly increased in plants exposed to BLED light at a glycine concentration of 80 mg L^{-1} (Fig. 8).

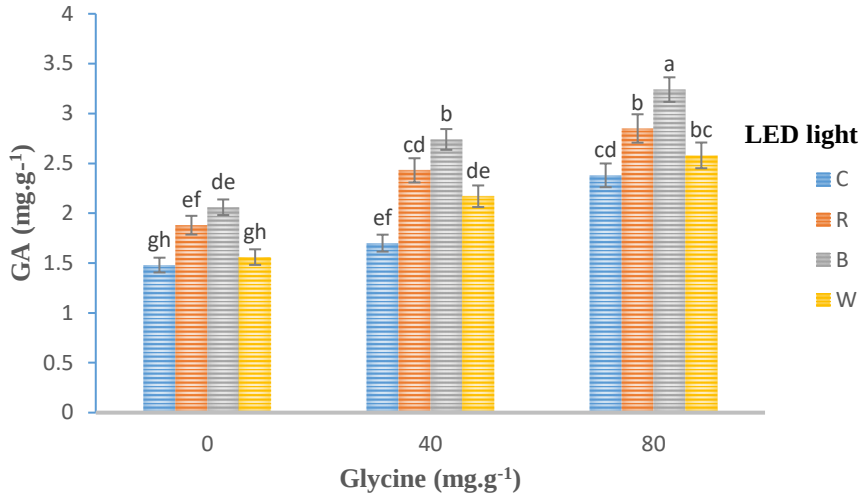

Fig. 6. Effects of glycine on GA in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test (P<0.05).

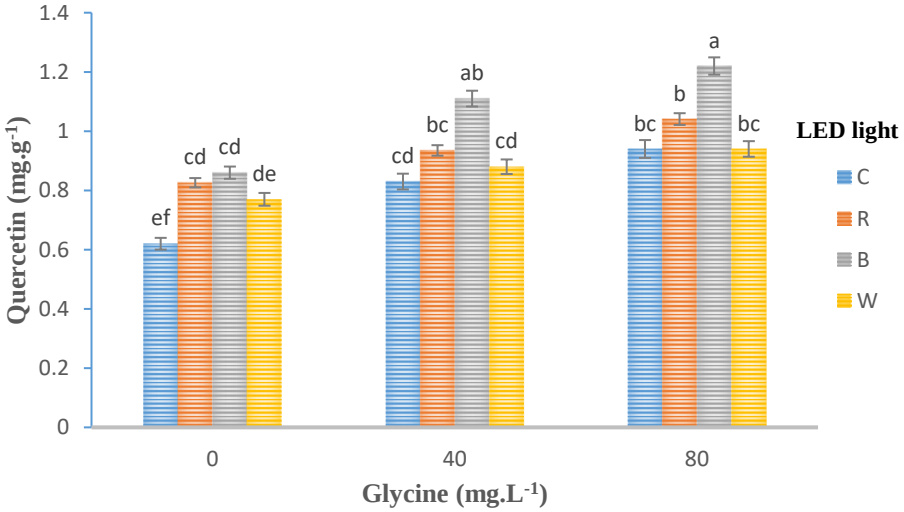

Fig. 7. Effects of glycine on quercetin in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test (P<0.05).

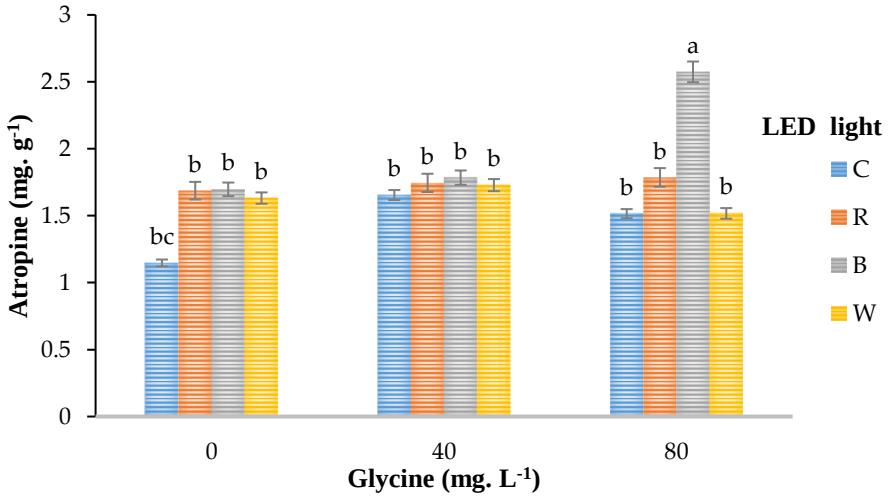

Fig. 8. Effects of LED light colors on atropine in *H. niger* L. plants stimulated with glycine. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test (P<0.05).

Our study revealed that LED lighting in *H. niger* L., compared to control (C) conditions, increased scopolamine levels. The highest scopolamine accumulation (22.61 mg g^{-1}) was observed in plants grown under BLED light with a glycine concentration of 40 mg L^{-1}. In contrast, the lowest scopolamine levels (2.711 mg g^{-1}) were detected in plants grown under WLED light with 80 mg L^{-1} glycine (Fig. 9).

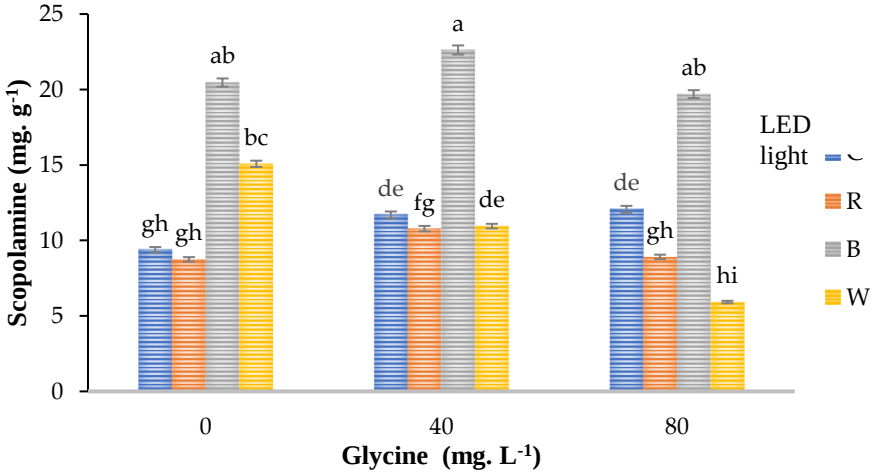

Fig. 9. Effects of glycine on scopolamine in *H. niger* L. plants stimulated with LED light colors. LED, C, B, R, and W refer to light color, normal light conditions, blue, red, and white, respectively. Whiskers indicate standard deviation. Values marked with the same letter do not differ significantly according to Duncan's multiple range test ($P<0.05$).

Heatmap of the average tropane alkaloid accumulation in dry weight of plants under LED light conditions

In various studies, Heatmap chart analysis is used as complementary data to illustrate the effects of treatments on different indices (Fig. 10). Heatmap data from this experiment highlighted the positive effects of LED light and glycine on several traits. Notably, the effects of glycine and LED lights were evident on root fresh matter ratio (FMR) and dry matter ratio (DMR) in *H. niger* L. (Fig. 10). The highest correlations for these two indices were observed under BLED light, with glycine at 40 and 80 mg L^{-1}, reaching 57.6% and 56.5% more than that of the control samples, respectively. Additionally, Heatmap data indicated that the highest levels of scopolamine and atropine accumulation were achieved under BLED light with 40 and 80 mg L^{-1} glycine.

Discussion

In recent years, significant efforts have been made to study the effects of different light spectra on the physiological processes and metabolism of various medicinal plants (Bantis et al., 2018). Successful production of medicinal plants is often achieved by optimizing biochemical parameters, where a higher TPC is usually associated with an enhanced ability to scavenge ROS. This is crucial for mitigating the adverse effects of environmental stress (Samuolienė et al., 2012; Heydarnajad Giglou and Torabi Giglou, 2023).

Our study demonstrated that yield efficiency, particularly DM in leaves, increased under BLED lighting combined with glycine at concentrations of 40 and 80 mg L^{-1} (Table 2). Similar results have been reported in other studies, where plant biomass increased under BLED conditions (Kong et al., 2016; Feijó et al., 2009; Chen et al., 2016). Moreover, glycine promoted plant height, and when combined with BLED, it significantly enhanced plant height compared to control plants and other LED light treatments (Table 2), as corroborated by previous research (Kopsell et al., 2016).

Researchers have suggested that ChlF parameters are an effective tool for detecting damage to the photosynthetic apparatus before visible morphological changes occur, making it a reliable indicator of light stress (Abidi et al., 2013). In our study, the F0 value increased under WLED light compared to the control, while Fm and Fv values increased under BLED conditions. This suggests that the response of chlorophyll fluorescence is dependent on the LED light color, potentially indicating plant stress (Kuo et al., 2015).

Interestingly, no significant changes in leaf color under LED lighting were observed in this study, which differs from the findings of other research. Based on a*, b*, and L* indexes, our results showed distinct changes in leaf color during different growth stages of *H. niger*, with BLED leading to a reduction in greenness and an increase in yellowness (Table 5). Previous studies have suggested that a combination of blue and red

LEDs can enhance carotenoid content, which is likely due to the dynamic interaction between colored LEDs and photosynthetic pigments (Kong et al., 2016; Feijó et al., 2009). Our results also indicated that glycine preserved leaf greenness by reducing the a* index, suggesting that the increase in green coloration is dependent on glycine concentration (Table 5).

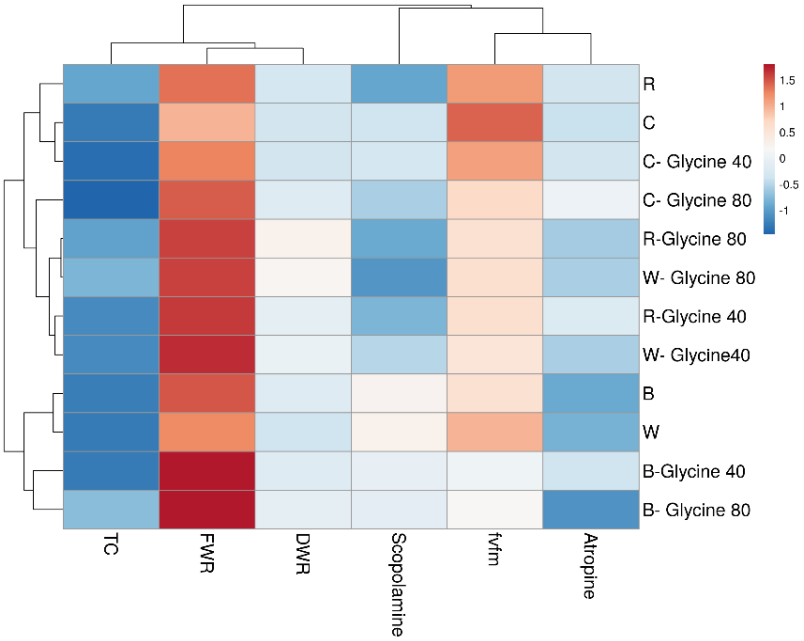

Fig. 10. Heatmap of average of tropane alkaloid accumulation in dry weight of plants under LED light conditions. Rows represent tropane alkaloid accumulation in dry weight and columns the various treatments expressed (glycine at 0, 40, and 80 mg L-1 concentration), C, B, R, and W refer to normal light conditions, blue, red, and white, respectively. FMR and DMR refer to fresh weight and dry weight of roots, respectively.

This study also investigated the effects of LED lighting and glycine on the production of tropane alkaloids, TPC, TFC, TAC, and TC in *H. niger*. It was found that both LED light and glycine significantly influenced secondary metabolite production. Different LED light colors not only supply energy for photosynthesis but also act as environmental signals that trigger various physiological responses in plants (Dhiman et al., 2018). During the growth of medicinal plants, LED lights can act as elicitors, enhancing SM production (Dhiman et al., 2018; Al Murad et al., 2021). Khalighi et al. (2021) also reported that plants grown under red and blue light (R) exhibited increased levels of total phenol (198.30%), soluble carbohydrates (65.31%), and essential oil (50.26%) compared to those grown under white light.

Amino acids, including glycine, are vital nitrogen sources for the synthesis of proteins and enzymes (Fawzy et al., 2012; Rouphael et al., 2017). Various studies have reported that amino acids enhance metabolite production (Colla et al., 2017), promote growth, and stimulate root development, which can improve water and nutrient uptake, thereby increasing productivity and yield (Khattab et al., 2016). When applied through the root system or via foliar feeding, amino acids improve the absorption and concentration of leaf nutrients (Mohammadipour et al., 2019).

Our results demonstrated that specific LED light colors, particularly BLED, combined with glycine at concentrations of 40 and 80 mg L^{-1}, increased the TA content in the studied plants. Additionally, the highest accumulations of scopolamine and atropine were observed under BLED when glycine was applied at 40 and 80 mg L^{-1} (Figs. 8 and 9). Glycine, being a rich source of nitrogen with a small molecular size, is likely easily absorbed by the plant during foliar application, and is utilized in the biosynthesis of TA (Ng et al., 2016). This could explain the observed increase in scopolamine and atropine levels in the plant.

To our knowledge, no previous studies have specifically investigated the impact of glycine on the accumulation of scopolamine (Fig. 9) and atropine (Fig. 8) in plants. However, previous research has shown that the application of amino acids, such as glycine, can have beneficial effects on both yield and the quality of SMs in medicinal plants (Karppinen et al., 2007; Fahimi et al., 2016; Souri et al., 2019).

Conclusions

This study aimed to investigate the effects of LED lighting and glycine on secondary metabolite accumulation, particularly total alkaloids, including scopolamine and atropine, in *H. niger* L. plants. The results demonstrated that LED lighting played a vital role in all stages of plant growth, especially in enhancing secondary metabolite production. Glycine, when used in combination with BLED, significantly increased secondary metabolite accumulation and helped mitigate the adverse effects of light stress, leading to improvements in both dry matter and fresh matter. The highest accumulations of scopolamine and atropine were observed under BLED when combined with glycine at concentrations of 40 and 80 mg L^{-1}. Furthermore, plants treated with 40 mg L^{-1} glycine under BLED exhibited the highest total antioxidant capacity. In terms of total phenolic content and total antioxidant capacity accumulation, BLED was found to be more effective than RLED in response to glycine foliar application on *H. niger* L.

Conflict of Interest

The authors indicate no conflict of interest in this work.

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