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Pre-harvest UV-B radiation during the dark period enhances phenolic compounds in *Agastache rugosa* cultivated in a plant factory with artificial lighting

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Abstract: *Agastache rugosa* Kuntze is an aromatic medicinal plant valued for its phenolic compounds in East Asia. To address the limitations of field cultivation, this study evaluated the interactive effects of photosynthetic photon flux density (PPFD), electrical conductivity (EC), and nutrient solution types on *A. rugosa* growth, to explore its introduction into controlled environments. After determining conditions for a stable yield, specifically 250 μmol/m^2/s PPFD and 2.5 dS/m EC using Hoagland solution, the effects of pre-harvest ultraviolet B (UV-B) radiation on secondary metabolite accumulation were examined. UV-B was applied during the dark period starting one week prior to harvest at 0.2 W/m^2 (4 or 8 h/day) or 0.4 W/m^2 (2 or 4 h/day), along with a control. Leaf rosmarinic acid (RA) content significantly increased in all UV-B treatments, with levels 44–66% higher than in the control, whereas tilianin and acacetin decreased by 17–25% and 61–73% under higher UV-B intensity or longer duration. Growth characteristics remained largely unaffected. These findings demonstrate the potential of UV-B radiation at 0.2 W/m^2 for 4 h/day during the dark period for enhancing RA content. Overall, this study highlights the feasibility of a pre-harvest UV-B application to enhance phenolic compounds in *A. rugosa* under optimized cultivation conditions.

Keywords: controlled environment agriculture; hydroponic production; nocturnal irradiation; rosmarinic acid; secondary metabolites

Agastache rugosa Kuntze is a perennial aromatic herb of the Lamiaceae family, widely cultivated and traditionally used in East Asia for ethnobotanical purposes (Bielecka et al. 2019). It is highly valued for secondary metabolites, including polyphenols such as rosmarinic acid (RA) and flavonoid glycosides like tilianin and acacetin (An et al. 2018). Most Lamiaceae species are rich in RA, which exhibits anti-inflammatory activities in humans. Tilianin demonstrates antihypertensive effects, whereas its biosynthetic precursor, acacetin, is a potent anti-cancer agent (Singh et al. 2020).

Commercial *A. rugosa* production under field conditions is limited to two cultivation cycles per

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year in temperate regions, limiting stable supply (Rosli et al. 2025). A plant factory with artificial lighting (PFAL) offers precise environmental control for year-round production of crops (Chun, Kozai 2002). In PFALs, light regulates both photomorphogenesis and secondary metabolism. For *A. rugosa*, increasing the photosynthetic photon flux density (PPFD) to 200 μmol/m^2/s improved biomass accumulation (Kim et al. 2017), and the white spectrum has enhanced RA and tilianin accumulation (Kim et al. 2018).

High-input PFALs necessitate maximised crop yields to justify investment costs. As hydroponics is commonly employed, nutrient solution management is critical for enhancing nutrient uptake efficiency (Shafi et al. 2019). The impacts of electrical conductivity (EC) levels or various solution types on *A. rugosa* growth have been investigated (Kim et al. 2017; Lam et al. 2020). As light-nutrient interaction influences plant development (Rosli et al. 2025), we investigated the interactive effects of PPFD, EC, and solution types to establish baseline cultivation conditions.

Functional crops require sufficient secondary metabolite accumulation without yield loss. Many studies have applied radiation outside the photosynthetically active radiation (PAR; 400–700 nm) to modulate key metabolites. Ultraviolet B (UV-B) radiation (280–320 nm) is the most energetic non-PAR radiation that plants commonly encounter. UV-B exposure is often associated with enhanced phenolics, which serve as protective agents against radiation (Weiland et al. 2023).

Most studies have applied UV-B during the photoperiod, while its dark-period use remains underexplored. The dark-period UV-B exposure stimulates UV-B receptors, potentially more effectively than with the photoperiod exposure (Ota et al. 2017). The dark-period UV-B has enhanced RA contents in greenhouse-grown perilla (Ota et al. 2017; Yoshida et al. 2021). As both the intensity and duration influence plant secondary metabolism (Kubota et al. 2017), assessing UV-B effects on individual secondary metabolites is essential.

We first examined the interactive effects of PPFD, EC, and solution type on the growth of *A. rugosa* cultivated in a PFAL. Upon establishing the optimal cultivation conditions for stable yield, we evaluated the effects of different intensities and durations of pre-harvest UV-B radiation applied during the dark period on the accumulation of key secondary metabolites.

MATERIAL AND METHODS

Plant materials and cultivation conditions during transplant production

A. rugosa seeds (Danong, Dongducheon, Korea) were germinated in polyurethane foam sponges (25 × 25 × 35 mm; Easyhydro, Hwacheon, Korea) and placed in 105-cell plug trays (Bumnong, Jeongeup, Korea). The germinated seedlings were grown under white LEDs (L-PEC, Jeonju, Korea) with 150 μmol/m^2/s and 16 h/day in a PFAL (Park et al. 2020b) (Figure 1).

Air temperature and relative humidity were maintained at 24 °C/20 °C and 65%/85% (photo-/dark periods), respectively, with slight modifications from the conditions described by Rosli et al. (2025). The seedlings were fertigated with either a modified Hoagland nutrient solution (hereafter 'Hoagland solution'; NO_3-N, 14.0 milliequivalents per litre

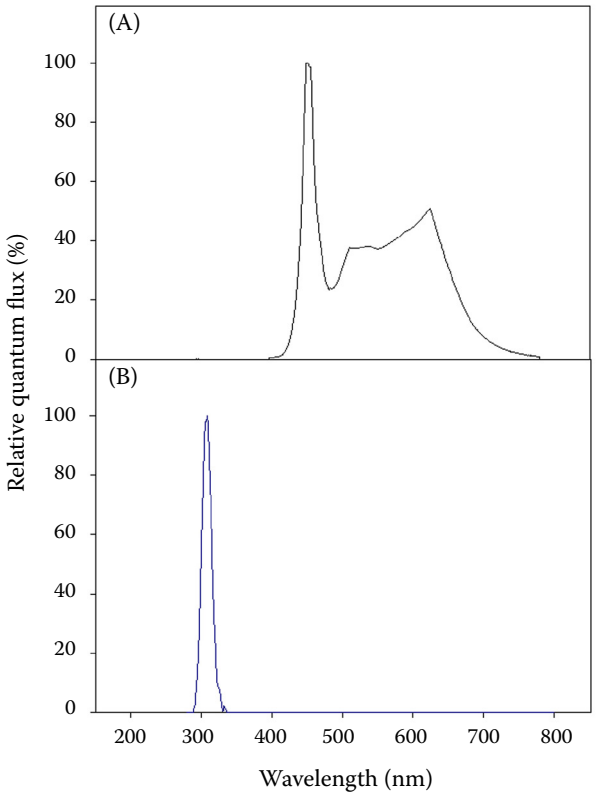

Figure 1. Spectral distribution of white (A) and ultraviolet B (B) light-emitting diodes used in a plant factory with artificial lighting

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(me/L); NH_4-N, 1.0 me/L; P, 3.0 me/L; K, 6.0 me/L; Ca, 8.0 me/L; Mg, 4.0 me/L; and S, 4.0 me/L; Hoagland, Arnon 1950) or the Yamazaki nutrient solution for lettuce (hereafter 'Yamazaki solution'; NO_3-N, 6.0 me/L; NH_4-N, 0.5 me/L; P, 1.5 me/L; K, 4.0 me/L; Ca, 2.0 me/L; Mg, 1.0 me/L; and S, 1.0 me/L; Yamazaki 1982), both with the same micronutrient composition (Fe, 3 mg/L; B, 0.5 mg/L; Mn, 0.5 mg/L; Zn, 0.05 mg/L; Cu, 0.02 mg/L; and Mo, 0.01 mg/L). The nutrient solution was delivered through a recirculating deep flow technique system and maintained at pH 6.0 and EC 1.5 dS/m during the transplant production phase using an automated control system, with the reservoir replenished once a week to maintain stable solution conditions.

Experimental treatments

Cultivation condition experiment. The photoperiod, air temperature, and relative humidity were maintained as in the transplant production phase. Eight treatment combinations were applied for three weeks after transplanting, including two PPFD levels (150 or 250 μmol/m^2/s), two EC levels (1.5 or 2.5 dS/m), and two solution types (Hoagland or Yamazaki solutions). Ten transplants with six to eight true leaves were transplanted onto polystyrene foam boards (planting density: 38.9 plants/m^2; GAFATEC, Anseong, Korea) for each treatment and subjected to the respective conditions.

Pre-harvest UV-B radiation experiment. According to the determined conditions, seedlings were fertigated with Hoagland solution during the transplant production phase, and the other environmental conditions were maintained. Transplants with six to eight true leaves were transplanted onto polystyrene foam boards (planting density: 33.3 plants/m^2; GAFATEC) with a slightly lower density for minimal shading of the lower leaves (Figure 2). The plants were cultivated for three weeks after transplanting under 250 μmol/m^2/s, and the same solution at 2.5 dS/m was supplied.

Pre-harvest UV-B radiation treatments were applied using UV-B LEDs (peak wavelength = 310 nm; L-PEC) (Figure 1). Treatments began one week before harvest and continued until harvest, applied at the beginning of each dark period, immediately after the photoperiod. UV-B was applied under combinations of two intensities and three durations: 0.2 W/m^2 for 4 or 8 h/day, and 0.4 W/m^2 for 2 or 4 h/day, along with a non-irradiated control (Figure 3). These treatments corresponded to two UV-B dosages (2.88 and 5.76 kJ/m^2/day). UV-B intensity was measured at the surface of the polystyrene foam board using a radiometer (VLX-3 W; Vilber-Lourmat, Torcy, France) equipped with a UV-B sensor (CX-312; Vilber-Lourmat).

Measurements

Growth characteristics. Fresh and dry weights of leaves, stems, and roots; plant height; number of nodes; leaf area; and relative chlorophyll content (SPAD value) were measured three weeks after transplanting, using 10 plants per treatment for the cultivation condition experiment, and five plants per treatment for the UV-B experiment, in which fresh and dry weights of inflorescences were also measured. After separating the shoots and roots, leaves longer than 2 cm and inflorescences longer than 1 mm were classified accordingly and weighed, and the remaining above-ground part was considered the stem. Dry weights were obtained by drying the samples in an oven at 80 °C for one week. Plant height was measured from the top surface of the polyurethane foam sponge to the shoot apical meristem. Nodes longer than 5 mm were counted. The total leaf area per plant was measured using a leaf area meter (LI-3100, LI-COR). SPAD values were averaged from the three longest leaves of each plant using a chlorophyll meter (SPAD-502, Konica Minolta, Sakai, Japan).

Secondary metabolite contents. Three plants were randomly selected from each replicate three weeks after transplanting, resulting in a total of nine



Figure 2. *Agastache rugosa* plants cultivated on polystyrene foam boards (planting density: 33.3 plants/m^2) for pre-harvest ultraviolet-B radiation treatments in a plant factory with artificial lighting

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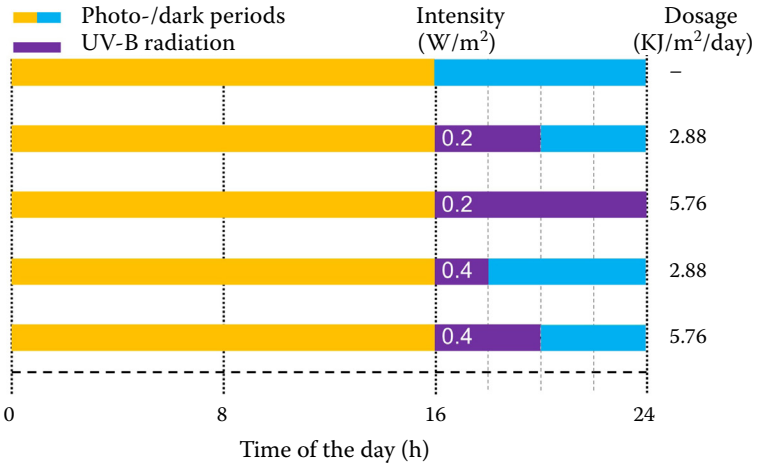

Figure 3. Schematic diagram of ultraviolet B (UV-B) intensity, exposure duration, and total dosage applied in the pre-harvest UV-B radiation treatments; UV-B radiation was applied during the dark period for one week prior to harvesting

biological samples per treatment. The whole leaves of each plant were sampled, immediately frozen in liquid nitrogen, and freeze-dried using a freeze dryer (FD8512, IlShinBioBase, Dongducheon, Korea) for one week. The dried leaves were then ground to a fine powder using a mortar and pestle, and 0.1 g of the powder was suspended in 1.5 mL of 80% (v/v) methanol with vortexing. The samples were sonicated for 60 min in an ultrasonic bath (JAC Ultrasonic 4020, KODO Technical Research, Hwaseong, Korea), followed by centrifugation at 12 000 × *g* for 10 min at 4 °C to collect the supernatant. The supernatant was filtered through a nylon syringe filter (0.45 µm pore size, PALL, NY, USA) and used for high-performance liquid chromatography (HPLC) analysis to quantify the RA, tilianin, and acacetin concentrations as described by Kim et al. (2018).

Statistical analysis

A non-replicated blocked design with 10 individual plants per treatment was used in the first experiment. The Statistical Analysis System (SAS; version 9.4, SAS Institute Inc., Cary, NC, USA) was used to perform a three-way analysis of variance (ANOVA) based on the Type III sum of squares, evaluating the main and interactive effects. In a second experiment with UV-B radiation, a completely randomised block design with three replicates was used, with each treatment within a replicate comprising eight individual plants. Differences among means were determined using one-way ANOVA. Mean comparisons were performed using Duncan's multiple range test at $P < 0.05$.

RESULTS

The main effect of PPFD was significant for all parameters except plant height, while the solution type-EC interaction affected all parameters except the SPAD values (Table 1). Three-way interaction effects were found on the fresh leaf weight and dry weight of all plant organs. Fresh leaf weight was the greatest under 2.5 dS/m and 250 µmol/m^2/s with the Hoagland solution (10.7 g), and under 1.5 dS/m and 250 µmol/m^2/s with the Yamazaki solution (10.5 g). Except under the conditions of 2.5 dS/m with the Yamazaki solution, both fresh leaf weight and fresh and dry stem weights were significantly enhanced by the higher PPFD. Fresh and dry root weights increased at the higher PPFD, with the highest values observed under 1.5 dS/m and 250 µmol/m^2/s with the Yamazaki solution. Plant height was the greatest under 2.5 dS/m with the Hoagland solution, regardless of the PPFD conditions, though its main effect was not significant. The number of nodes was significantly affected by all the main effects and interactions, except for the main effect of solution type. Leaf area significantly increased at higher PPFD with the Hoagland solution, and three-way interactive effects were observed.

Most growth parameters showed no significant differences among UV-B treatments (Table 2). Dry stem weight was significantly higher in the 0.4 W/m^2 for 2 h/day treatment, and the number of nodes increased significantly in all UV-B treatments except the 0.4 W/m^2 for 4 h/day treatment, compared to the control. No visible damage to leaves was observed in any of the UV-B treatments (Figure 4). All UV-B treatments significantly increased the

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Table 1. Growth characteristics of *Agastache rugosa* measured three weeks after transplanting as affected by the nutrient solution type, electrical conductivity (EC), and photosynthetic photon flux density (PPFD)

Nutrient solution type (N)	EC (dS/m) (E)	PPFD (μmol/m^2/s) (P)	Fresh weight (g)			Dry weight (g)			Plant height (cm)	No. of nodes	Leaf area (cm^2)	SPAD value
			leaf	stem	root	leaf	stem	root				
Hoagland	1.5	150	6.3^{d}	5.5^{e}	5.3^{d}	1.2^{e}	0.66^{f}	0.73^{d}	36.6^{c}	7.3^{c}	507.5^{d}	50.2^{c}
		250	9.4^{b}	8.4^{bc}	8.5^{c}	2.28^{c}	1.07^{c}	0.99^{bc}	40.5^{b}	8.7^{ab}	620.3^{ab}	55.1^{bc}
	2.5	150	6.8^{d}	7.2^{cd}	6.2^{d}	1.48^{e}	0.91^{de}	0.75^{d}	44.6^{a}	9.0^{a}	539.3^{cd}	50.2^{c}
		250	10.7^{a}	10.3^{a}	8.6^{c}	3.10^{a}	1.43^{a}	1.11^{b}	44.6^{a}	9.0^{a}	667.1^{a}	61.3^{ab}
Yamazaki	1.5	150	8.7^{bc}	9.0^{b}	8.6^{c}	1.82^{d}	1.03^{cd}	0.91^{c}	42.8^{ab}	8.4^{b}	622.4^{ab}	55.1^{bc}
		250	10.5^{a}	10.5^{a}	12.1^{a}	2.72^{b}	1.29^{b}	1.28^{a}	42.2^{ab}	8.5^{ab}	686.7^{a}	61.6^{a}
	2.5	150	8.2^{c}	7.2^{cd}	7.5^{c}	1.87^{d}	0.84^{e}	0.89^{c}	41.5^{ab}	8.4^{b}	582.6^{bc}	56.2^{a-c}
		250	8.8^{bc}	6.7^{d}	10.2^{b}	2.43^{bc}	0.87^{e}	1.10^{b}	36.4^{c}	8.4^{b}	516.1^{cd}	60.4^{ab}
N			**	ns	***	*	ns	***	ns	ns	ns	**
E			ns	ns	ns	*	ns	ns	ns	***	ns	ns
P			***	***	***	***	***	***	ns	**	***	***
N × E			***	***	**	***	***	**	***	***	***	ns
N × P			***	***	ns	***	***	ns	**	**	***	ns
E × P			ns	ns	ns	ns	ns	ns	*	**	ns	ns
N × E × P			*	ns	ns	**	*	*	ns	**	*	ns

Means within columns followed by different letters indicate statistically significant differences based on Duncan's multiple range test at $P < 0.05$; asterisks indicate significance in the three-way analysis of variance evaluation on the effects of nutrient solution types, ECs, and PPFDs: *,**,***$P < 0.05$, 0.01, 0.001; ns, not significant

RA content in leaves (8.0–9.2 mg/g DW) relative to that in the control (5.5 mg/g DW) (Figure 5A). Although the differences among UV-B treatments were not statistically significant, all treatments consistently induced a marked increase of approximately 44–66% over the control. Significantly lower concentrations of tilianin were observed in the 0.2 W/m^2 for 8 h/day, 0.4 W/m^2 for 2 h/day, and 0.4 W/m^2 for 4 h/day treatments (< 1.2 mg/g DW), compared to the control (1.4 mg/g DW) (Figure 5B). Similarly, acacetin content was maintained only in the 0.2 W/m^2 for 4 h/day treatment

Table 2. Growth characteristics of *Agastache rugosa* as affected by the intensity and exposure duration of pre-harvest ultraviolet B radiation applied during the dark period

Intensity (W/m^2)	Duration (h/day)	Fresh weight (g)				Dry weight (g)				Plant height (cm)	No. of nodes	Leaf area (cm^2)	SPAD value
		leaf	stem	root	inf	leaf	stem	root	inf				
–	–	9.4	7.7	8.1	0.15	2.07	1.05^{b}	0.72	0.03	36.0	9.2^{b}	472.3	57.6
0.2	4	9.5	8.0	8.2	0.17	2.09	1.13^{ab}	0.75	0.04	36.2	9.9^{a}	466.3	58.6
0.2	8	9.5	7.8	8.2	0.13	1.99	1.04^{b}	0.73	0.03	36.1	9.6^{a}	475.8	57.1
0.4	2	10.4	8.9	9.2	0.23	2.15	1.25^{a}	0.84	0.04	38.3	9.9^{a}	515.1	58.3
0.4	4	10.3	8.6	8.8	0.21	2.10	1.16^{ab}	0.78	0.04	36.4	9.1^{b}	511.0	58.0
		ns	ns	ns	ns	ns	*	ns	ns	ns	***	ns	ns

inf – inflorescence; means within columns followed by different letters indicate statistically significant differences based on Duncan's multiple range test at $P < 0.05$; asterisks indicate significance in the analysis of variance evaluation: *,*** $P < 0.05$, 0.001; ns – not significant

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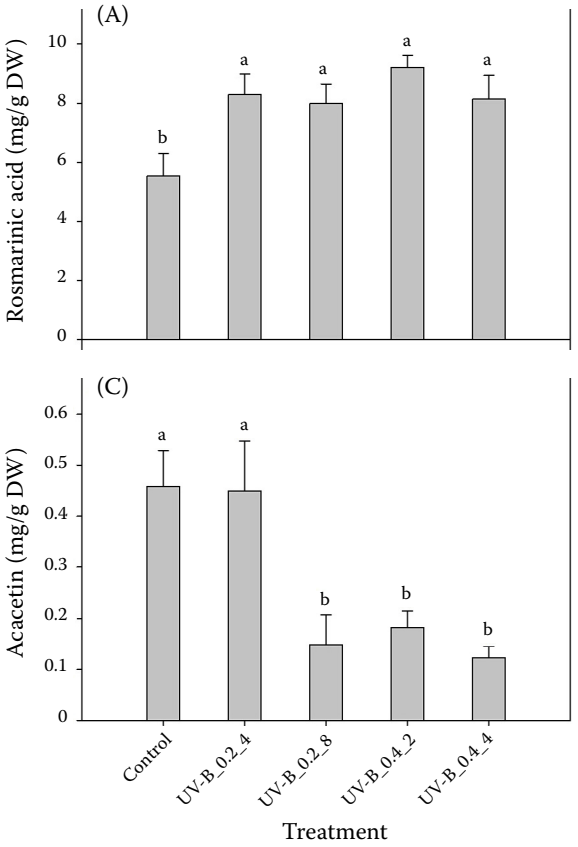
Figure 4. Growth of *Agastache rugosa* as affected by the intensity and exposure duration of pre-harvest ultraviolet B (UV-B) radiation applied during the dark period

Treatments were as follows: Control – no UV-B radiation; UV-B_0.2_4 – 0.2 W/m^2 for 4 h/day; UV-B_0.2_8 – 0.2 W/m^2 for 8 h/day; UV-B_0.4_2 – 0.4 W/m^2 for 2 h/day; UV-B_0.4_4 – 0.4 W/m^2 for 4 h/day

(0.45 mg/g DW), compared to that in the control (Figure 5C). The other UV-B exposure conditions substantially decreased acacetin concentrations to levels approximately 73% lower than those in the control. The acacetin contents were more substantially reduced than the tilianin contents under UV-B treatments, except in the 0.2 W/m^2 for 4 h/day treatment, compared to those in the control.



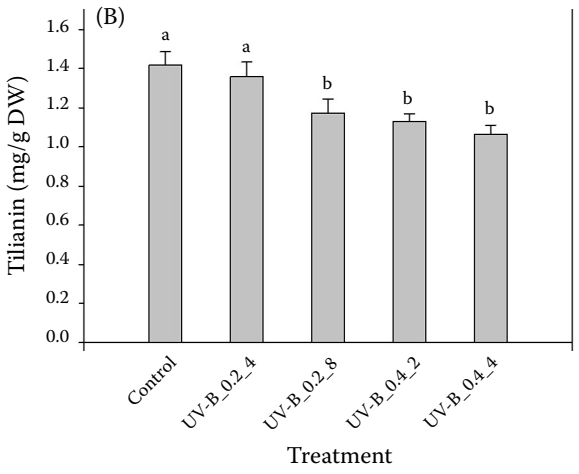


Figure 5. Rosmarinic acid (A), tilianin (B), and acacetin (C) contents in the leaves of *Agastache rugosa* as affected by the intensity and exposure duration of pre-harvest ultraviolet B (UV-B) radiation applied during the dark period

Treatments were as follows: Control – no UV-B radiation; UV-B_0.2_4 – 0.2 W/m^2 for 4 h/day; UV-B_0.2_8 – 0.2 W/m^2 for 8 h/day; UV-B_0.4_2 – 0.4 W/m^2 for 2 h/day; UV-B_0.4_4 – 0.4 W/m^2 for 4 h/day

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DISCUSSION

In the present study, 250 μmol/m^2/s PPFD enhanced leaf weights, as reflected by the significant main effect of PPFD on yield. Although lower than typical greenhouse levels, the PPFD falls within the range commonly used for indoor cultivation of leafy medicinal herbs. Biomass of *A. rugosa* has previously been shown to increase at approximately 200 μmol/m^2/s (Kim et al. 2017), supporting our light conditions. While the sole main effect of EC was significant only for the dry leaf weight and number of nodes, a significant three-way interaction influenced key yield traits, including fresh and dry leaf weights and leaf area. These interactions indicate that nutrient availability and PPFD jointly regulate leaf biomass accumulation, emphasising that EC optimisation should be adjusted according to PPFD conditions.

Increased EC within a proper range may benefit both biomass and secondary metabolite accumulation (Rosli et al. 2025). Elevated EC from 0.5 to 2.5 dS/m improved the antioxidant capacity and biomass in *Crepidiastrum denticulatum* under high PPFD, with the most pronounced effects observed at EC ≥ 2.0 dS/m (Park et al. 2016). Similarly, our findings suggest that an EC of at least 2.5 dS/m supports biomass production, likely by maintaining nutrient uptake and osmotic balance under higher irradiance. Although the Yamazaki solution at 1.5 dS/m also promoted plant growth, it resulted in lower dry leaf weight, suggesting relatively suboptimal nutrient supply. Because leaves exhibit the highest antioxidant activity in *A. rugosa* (Desta et al. 2016), maximising leaf biomass was prioritised. Therefore, 2.5 dS/m with the Hoagland solution and 250 μmol/m^2/s was selected as the optimal condition for subsequent UV-B treatments.

Previous studies in Lamiaceae species have shown that low UV-B doses can enhance phenolic accumulation, whereas higher doses often suppress growth. Dark-period UV-B at 0.08 to 0.12 W/m^2 for 3 h/day over three weeks reduced leaf size and altered pigmentation in perilla (Ota et al. 2017). In contrast, Yoshida et al. (2021) demonstrated that UV-B at 0.05 W/m^2 for 3 h/day during the dark period over five weeks significantly increased RA accumulation in perilla. However, higher intensities (0.21 and 0.88 W/m^2) suppressed biomass accumulation in PFAL-grown perilla (Nishimura et al. 2008). Based on these findings on related Lamiaceae species, UV-B treatments were designed to provide a moderate stress stimulus sufficient to activate metabolism without compromising growth, with total exposure limited to one week. The absence of morphological damage and unchanged growth parameters across treatments confirms that yield stability was maintained.

RA accumulation was predominant among compounds, consistent with a previous report on *A. rugosa* (Lam et al. 2020). The treatment of 0.2 W/m^2 for 4 h/day (total dose: 2.88 KJ/m^2/day) significantly increased RA content. In contrast, higher intensities (0.4 W/m^2 for 2 or 4 h/day) or prolonged durations (0.2 W/m^2 for 8 h/day; 0.4 W/m^2 for 4 h/day) may induce a metabolic shift from secondary metabolite synthesis toward stress acclimation under higher UV-B doses, reducing tilianin and acacetin accumulation. Comparable dose-dependent responses have been reported in other medicinal crops (Park et al. 2020a), supporting the concept that UV-B regulation of secondary metabolism depends on maintaining stress within a crop-specific tolerable threshold.

UV-B radiation is also unlikely to induce photoperiodic flowering responses (Takahashi et al. 2025), consistent with unchanged inflorescence weight. Because RA levels in *A. rugosa* decline during flowering (Lee et al. 1999), applying pre-harvest UV-B radiation during the dark period appears to balance stress-induced metabolite enhancement with maintenance of vegetative growth, thereby improving phytochemical consistency without compromising yield.

CONCLUSION

The growth of *A. rugosa* was significantly influenced by the interactive effects of PPFD, EC, and solution type, suggesting that 250 μmol/m^2/s PPFD combined with 2.5 dS/m EC using the Hoagland solution represents an optimal cultivation condition. Application of UV-B at 0.2 W/m^2 for up to 4 h/day during the dark period one week prior to harvest significantly increased RA content. However, deceased tilianin and acacetin concentrations by higher UV-B intensity or longer exposure durations underscore the importance of appropriate dosage thresholds. Collectively, integrating optimised light and nutrient regimes with modulated pre-harvest UV-B supplementation offers a viable strategy for standardising medicinal herb production in controlled environment agriculture.

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