horticulturae

MDPI

Article

Effect of Pre-Harvest Intermittent UV-B Exposure on Growth and Secondary Metabolites in *Achyranthes japonica* Nakai Microgreens in a Vertical Farm

Ye Lin Kim [1], Moon-Sun Yeom [2], Han-Sol Sim [1], Ga Oun Lee [1], In-Je Kang [3], Gyu-Sik Yang [3], Jae Gil Yun [3] and Ki-Ho Son [1,3,*]

[1] Department of GreenBio Science, Gyeongsang National University, Jinju 52725, Republic of Korea; dpflskol@naver.com (Y.L.K.); thfdl2828@naver.com (H.-S.S.); riyung@naver.com (G.O.L.)
[2] Department of Horticultural Science, Chungbuk National University, Cheongju 28644, Republic of Korea; msy1023@cbnu.ac.kr
[3] Division of Horticultural Science, Gyeongsang National University, Jinju 52725, Republic of Korea; nowkang123@naver.com (I.-J.K.); yrbtlr@naver.com (G.-S.Y.); jgyun@gnu.ac.kr (J.G.Y.)
* Correspondence: sonkh@gnu.ac.kr

Abstract: *Achyranthes japonica* Nakai (AJN) is a medicinal plant known to be beneficial for the joints. Since it takes at least two years from sowing to harvesting in an open field, new AJN cultivation strategies are needed to shorten the production period and improve quality. In this study, high-quality AJN is produced as microgreens in a vertical farm using a commercial ginseng soil mix (Myeongpum-Insamsangto, Shinsung Mineral Co., Ltd., Goesan, Republic of Korea) and controlled environmental conditions. The cultivation conditions included a temperature of 23 ± 2 °C, relative humidity of 50 ± 10%, and a photosynthetic photon flux density of 170 ± 15 μmol·m^{-2}·s^{-1}. Pre-harvest intermittent UV-B exposure, with an intensity of 1.0 ± 0.3 W/m^{-2}, was applied for one day before harvest to evaluate its effects, using controlled environmental conditions in the vertical farm. Ultraviolet-B (UV-B) irradiation increases secondary metabolite levels in plants; however, the effect of UV-B on 20-hydroxyecdysone (20E), an indicator of AJN, is unclear. Therefore, we aimed to investigate whether UV-B treatment of AJN microgreens affected growth and secondary metabolites. The treatment group was set to 12 h of continuous UV-B treatment during the day, two 6 h UV-B treatments, and four 3 h UV-B treatments to confirm the effectiveness of regular and intermittent treatment and recovery. Short-term UV-B treatment before harvesting increased phenols, flavonoids, antioxidant capacity, and 20E levels without affecting AJN biomass. The intermittent 6 h UV-B irradiation with a 6 h recovery time stimulated 20E content by approximately 1.4 times compared to the control. These study findings indicate that short-term UV-B treatment before harvesting, an appropriate recovery time, and intermittent UV-B exposure are more effective at increasing 20E content than continuous treatment. This approach provides a promising strategy for improving the nutritional and health benefits of AJN microgreens in vertical farming systems.

Keywords: ultraviolet light; plant stress; medicinal plant; vertical farm; 20-hydroxyecdysone; pre-harvest

Citation: Kim, Y.L.; Yeom, M.-S.; Sim, H.-S.; Lee, G.O.; Kang, I.-J.; Yang, G.-S.; Yun, J.G.; Son, K.-H. Effect of Pre-Harvest Intermittent UV-B Exposure on Growth and Secondary Metabolites in *Achyranthes japonica* Nakai Microgreens in a Vertical Farm. *Horticulturae* **2024**, *10*, 1040. https://doi.org/10.3390/horticulturae10101040

Academic Editor: Jiri Gruz

Received: 5 August 2024
Revised: 9 September 2024
Accepted: 24 September 2024
Published: 30 September 2024

1. Introduction

Achyranthes japonica Nakai (AJN) is distributed in East Asia, particularly in the Republic of Korea, Japan, and China, and belongs to the *Achyranthes* genus within the Amaranthus family [1].

The AJN plant is used as an herbal medicine that can be used for medicinal purposes as well as an additive to healthy, functional foods [2]. Phytoecdysteroids are plant sterols that have the same or similar structure to that of insect molting hormones. Among them, ten species belonging to the genus *Achyranthes* and contain compounds such as

20-hydroxyecdysone (20E), polypodine B, and inokosterone [3]. Notably, the presence and abundance of 20E in AJN serves as a crucial indicator and key marker of the plant's medicinal value [4]. Furthermore, 20E causes vasodilation, increases blood flow, and affects skeletal muscle circulation [5]. Given the traditionally long cultivation period of over two years for AJN in open fields, this study investigated whether the microgreen form of AJN, characterized by a significantly shorter production cycle, could still yield high levels of bioactive compounds, specifically 20E [6]. By exploring this faster and more efficient cultivation method, we aimed to assess if the medicinal value of AJN could be maintained or enhanced.

The growth of medicinal plants as functional materials and microgreens in vertical farms is emerging as an innovative method [7]. Vertical farming is a crop-production strategy that uses controlled environmental agriculture. This approach allows for the year-round cultivation of crops independent of seasonal changes and adverse weather conditions, thereby bolstering food security. However, vertical farming systems require marked initial investments in technology and infrastructure, such as light-emitting diode (LED) lighting and environmental control systems [8]. Therefore, it is crucial for vertical farms to produce crops that proliferate and offer high added value [9]. This includes high-value crops like leafy greens, herbs, and medicinal plants, which have shorter growth cycles and can be harvested multiple times per year, maximizing the return on investment and making the best use of the controlled environment.

Additionally, in vertical farming, cultivation methods are diverse, including hydroponics and soil-based cultivation [10]. While medicinal plants are traditionally cultivated in soil, there is growing research interest in hydroponic cultivation, which offers advantages such as increased biomass and secondary metabolites [11] in vertical farming systems. These varied cultivation methods present novel opportunities for enhancing the productivity and quality of medicinal plants in vertical farming. Therefore, research on cultivation methods in vertical farming is becoming increasingly important as it offers avenues for improving the efficacy and safety of medicinal plants [12].

Microgreens are young seedlings, typically less than 3 weeks old, with two fully developed leaves along with their first true leaves [13]. These microgreens are valued not only for their delicate flavor but also for their dense nutritional profile, which includes higher concentrations of essential vitamins, minerals, and antioxidants compared to their mature counterparts. According to various studies, young seedlings and microgreens contain more phytonutrients than mature leaves [13]. One study found that cabbage and radish sprouts and seedlings had a higher glucosinolate content than mature plants [14]. Owing to their small size and compact cultivation methods, microgreens are usually produced in vertical farms to maximize the yield per unit of land area [15]. Changing the wavelengths of light in vertical farms affects the morphological, physiological, and biochemical characteristics of plants [16]. Previous studies indicate that blue LED lighting can enhance glucosinolate content in both broccoli sprouts [17] and pak choi microgreens [18]. Additionally, UV-A light has been observed to enhance the polyphenol content and antioxidant effects of pak choi microgreens [19]. Various horticultural species have been evaluated for their ability to produce microgreens [20], and light control may be beneficial for developing microgreens containing the desired nutritional and health-related compounds [21].

Light provides plants with energy for photosynthesis, while ultraviolet-B (UV-B) irradiation, in particular, significantly impacts the physiology of plant seedlings, including microgreens, influencing various physiological and biochemical processes [22]. UV-B is the most significant energy source reaching the Earth's surface in the solar spectrum (280–315 nm). Plants have evolved several defense systems to cope with natural UV-B stress. These defense mechanisms are particularly crucial during the vulnerable seedling stage, where UV-B treatment not only enhances stress tolerance but also promotes the accumulation of secondary metabolites, such as flavonoids and phenolic compounds [23]. Under stress, phenolics act as antioxidants, antimicrobials, UV-B blockers, and scavengers of reactive oxygen species (ROS), with their levels varying in UV-B-exposed plants based

on factors such as species, intensity, and duration [24]. Several studies have examined the effects of UV-B on plants, with a growing focus on increasing secondary metabolite levels by applying UV-B under controlled environmental conditions [17]. In addition, short-term (several days) UV-B irradiation was recently discovered to be potentially effective as a pre-harvest treatment for obtaining antioxidant-rich plant products [25]. Studies on UV-B stress have shown that the secondary metabolites of amaranth [26], ginseng [27], and buckwheat [28] increase with UV-B treatment. Although interest in the pre-harvest UV-B exposure of crops in indoor cultivation is increasing, research on its effect on the quality of microgreen functional foods is insufficient [22]. Therefore, pre-harvest UV-B treatment of microgreens and sprouts in vertical farms is considered an effective method for rapidly increasing plant bioactive compounds [29].

Therefore, this study aimed to assess the effects of pre-harvest UV-B treatment on the growth and bioactive compounds of AJN microgreen forms in vertical farms. However, it must be noted that this study primarily focused on soil-based cultivation within vertical farming systems. Nevertheless, future investigations will consider the productivity and potential of hydroponic cultivation systems.

2. Materials and Methods

2.1. Plant Materials

AJN seeds (Aram Seeds Co., Ltd., Seoul, Republic of Korea) with a weight of 3.0 g per 1000 seeds were used in this study; 1.0 g of seed (approximately 330 seeds) was used per treatment. AJN seeds were immersed in distilled water for three days before sowing. The seeds were sterilized using a modified version of Oyebanji's method, soaked in 70% ethanol for 30 s and rinsed thrice with distilled water [30]. The seeds were then sterilized with 20% NaOCl (Duksan Science Co., Ltd., Seoul, Republic of Korea) for 15 min and rinsed in distilled water four times.

2.2. Cultivation Conditions

For each treatment, 1 g of AJN seed was sown into a plastic tray (31.5 × 23 × 7.5 cm, L × W × H) filled with a commercial ginseng soil mix medium (Myeongpum-Insamsangto, Shinsung Mineral Co., Ltd., Goesan, Republic of Korea) in a cultivation room. Microgreen sowing and cultivation methods involved evenly distributing seeds onto the substrate and irrigating using bottom watering with 200 mL of nutrient solution. Germination was carried out in dark condition over four days, after which the light was turned on. The cultivation room was maintained at 23 ± 2 °C, 50 ± 10% relative humidity, and 170 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) using white LEDs over a 12 h photoperiod. PPFD levels were measured with a quantum sensor (Li-250A, Li-Cor, Lincoln, NE, USA), and temperature and humidity were monitored and regulated daily using a wireless data logger (TH2, Efento, Krakow, Poland). The plants were irrigated using Hoagland's nutrient solution (pH 6.0, EC 1.0 $dS \cdot m^{-1}$). The environmental conditions were established based on previous studies.

2.3. Light Treatment

The UV-B LEDs (TUNP-AG120-UV-B100%-23W-PA, Japan magnets, Suwa, Japan; peak 308 nm) were supplemented with 1.0 ± 0.3 $W \cdot m^{-2}$ (Figure 1B). UV-B levels were measured using LP 471 UV-B sensor supplied by PCE Instruments S.L. (Albacete, Spain). Seventeen-day-old AJN plants were irradiated with UV-B LEDs for one day before harvesting (harvested on day 18). The treatment groups were (1) continuous 12 h treatment (UV12/C1), (2) two 6 h intermittent treatments per light/dark cycle (UV06/C2), and (3) four 3 h intermittent treatments (UV03/C4). The cumulative UV-B time was equal to a total of 12 h. The control group (control) was not exposed to UV-B radiation (Figure 1A).

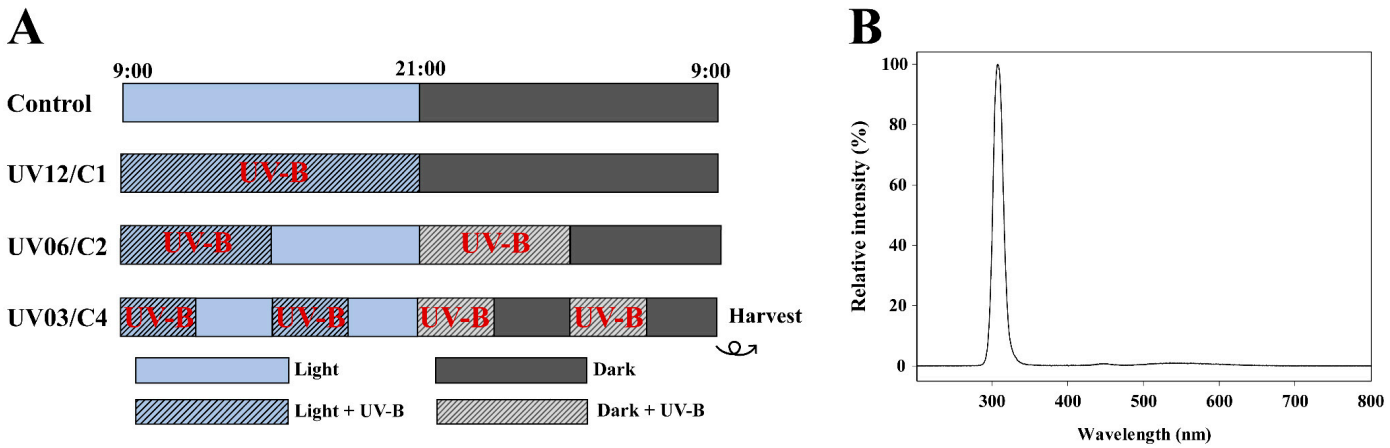

Figure 1. Treatments (**A**) and relative intensity (**B**) of UV-B LEDs used in this study. The periods of UV-B irradiation were from 9:00 to 21:00 (UV12/C1), twice in a 6 h cycle (UV06/C2), and four times in a 3 h cycle (UV03/C4). The energy of the UV-B was set to 1.0 ± 0.3 $W \cdot m^{-2}$ and was applied one day before harvest.

2.4. Determination of Biomass and Growth Characteristics

To compare AJN growth according to the UV-B treatment periods, the fresh weight (FW), dry weight (DW), shoot height, leaf and stem dry weight, leaf area, specific leaf area (SLA), and relative moisture content (RWC) were measured 18 d after sowing. The RWC and plant height were measured using 4 plants, while leaf and stem weight, leaf area, and SPAD values were measured using 10 plants. A digital scale (PAG214C, Ohaus Corp., Parsippany, NJ, USA) was used to measure FW and DW. An area meter (Li-3100, LICOR, Lincoln, NE, USA) was used to measure the leaf area, and SPAD values (SPAD-502Plus, Konica Minolta, Osaka, Japan) were measured using the largest true leaves. Samples were placed in a dry oven (EP20, KOTES, Co., Ltd., Seoul, Republic of Korea) at 55 °C for 72 h to determine DW. RWC was calculated using FW and DW [31], and SLA was calculated using leaf DW and leaf area [32], as follows:

$$\text{RWC (\%)} = \text{FW (g)} - \text{DW (g)}/\text{FW (g)} \times 100$$

$$\text{SLA (cm}^2/\text{g)} = \text{leaf area (cm}^2)/\text{leaf dry weight (g)}$$

2.5. Determination of Chlorophyll Fluorescence

The aerial parts of the AJN seedlings treated with different UV-B periods for one day were selected. As biological replicates, five plants were chosen from each treatment. After dark adaptation for 15 min, the Fv/Fm values of the true leaves were measured using a portable chlorophyll fluorometer (Chlorophyll Fluorometer, OS30P+, OPTI-SCIENCES. Inc., Hudson, NH, USA). Measurements were performed once every 3 h for the control, UV12/C1, UV06/C2, and UV03/C4 treatments.

2.6. Bioactive Compounds Analysis

Total phenolic content was measured using the modified Folin–Denis method [33]. Dried powder samples (0.015 g) were mixed with 1.5 mL of 80% acetone and sonicated for 15 min and stored at 4 °C for 12 h. The supernatant was then obtained after centrifugation (WiseSpin, CF-10, Daihan Scientific Co, Ltd., Wonju, Republic of Korea) at 25 °C and 13,500 rpm for 2 min. In a microcentrifuge tube, distilled water, 10% Folin–Ciocalteu reagent, the sample, and 7.5% Na_2CO_3 were added in that order and mixed. Each analyzed sample was placed in a water bath set at 45 °C for 15 min. After being cooled at room temperature for about 30 min, the absorbance of each sample was measured at 765 nm using a spectrophotometer (Libra S32, Biochrom Ltd., Cambridge, UK). The total phenolic concentration of the AJN microgreens was expressed as milligrams of gallic acid equivalent

per gram of DW AJN shoots. A standard curve was generated using different concentrations of gallic acid to quantify the phenolic content of the samples.

Flavonoid content was measured using a modified colorimetric method [34]. Sample extracts were prepared by incubating solutions of dry powder samples (1 g) and 20 mL 50% methanol (MeOH) at room temperature for 14 ± 2 h using a stirrer. Subsequently, 0.5 mL of the diluted extract was aliquoted into a test tube, and 1 mL of diethylene glycol and 0.01 mL of 1 N NaOH were added. The mixture was incubated in a bath set at a constant temperature of 37 °C for 1 h, and absorbance was measured at 420 nm using a spectrophotometer (UV-1800 240V, Shimadzu Corp., Kyoto, Japan). Flavonoid content was then determined using a standard curve prepared with rutin, and the results were expressed as milligrams of rutin equivalent per gram of dry weight (RE/DW) of *Achyranthes japonica* Nakai shoots.

2.7. Extraction and Determination of Antioxidant Properties

For the analysis of antioxidant properties, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and ferric reducing/antioxidant power (FRAP) were used. Dried powdered samples were used. Sample extracts were obtained by mixing 1 g of the sample powders with 20 mL of 50% methanol (MeOH) and stirring the solutions at room temperature for 14 ± 2 h. Slightly modified radical (DPPH and ABTS)-scavenging activity and FRAP evaluation methods were used [35]. A spectrophotometer (UV-1800 240V, Shimadzu Corp., Kyoto, Japan) was used to measure the absorbance of the in vitro activities.

DPPH radical-scavenging activity was assessed by measuring absorbance at 525 nm after adding 0.2 mL of each sample to 0.8 mL of 1.5×10^{-4} M DPPH solution for 30 min in the dark.

ABTS radical-scavenging activity was measured by mixing 7 mM $ABTS^{+}$ solution with 2.45 mM $K_2S_2O_8$ in a 1:3 ratio, and incubating in the dark for 14 ± 2 h. It was then diluted with methanol to obtain an absorbance of 0.7 ± 0.02 at 732 nm. After adding 0.1 mL of each sample extract to 0.9 mL of the $ABTS^{+}$ solution, the mixture was allowed to react for 3 min in the dark, and absorbance was measured.

The FRAP assay was performed by combining 5 mL each of 10 mM TPTZ and 20 mM $FeCl_3$ with 50 mL of 300 mM sodium acetate buffer (pH 3.6) to obtain a 10:1:1 ($v/v/v$) solution, which was pre-reacted at 37 ± 1 °C for 15 min to prepare the FRAP. Then, 0.05 mL of the sample extracts were mixed with 0.95 mL of the FRAP reagent and allowed to react at 37 ± 1 °C for 15 min. Absorbance was then measured at 590 nm.

Ascorbic acid and $FeSO_4 \cdot 7H_2O$ were used as standard materials to measure radicals (DPPH and ABTS) and FRAP, respectively. The results were expressed in mg/g using ascorbic acid and $FeSO_4 \cdot 7H_2O$ by inserting the calculation formula derived from the standard curve.

2.8. Liquid Chromatography-Mass Spectrometry (LC-MS/MS) Analyses of 20-Hydroxyecdysone

Dried samples (0.1 g) were extracted with 15 mL of 50% methanol using a sonicator for 10 min. Two microliters of each extract were then injected into a LIChrospher®100 RP-18 5 µm, end-capped (Merck Eurolab, Darmstadt, Germany) column connected to a Shimadzu Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan). The liquid chromatography system was coupled to a SCIEX QTRAP 4500 mass spectrometer equipped with a Turbo V Ion Source and a Turbo Ion Spray probe for electrospray ionization (SCIEX, Framingham, MA, USA) at the High-Tech Materials Analysis Core Facility (Gyeongsang National University, Jinju, Republic of Korea). The elution solvent was carried out with a gradient of solvent A (water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The solvent composition was adjusted from 20% A at 2 min to 90% B at 6 min with a flow rate of 0.5 mL/min. The MS/MS conditions were set as follows: ESI source ion spray voltage, 5500 V (positive ion mode); source temperature, 550 °C; 50 psi ion source gas (GS1), 60 psi ion source gas (GS2), 30 psi curtain gas (CUR).

2.9. Statistical Analysis

Data were analyzed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA) with variance analysis. Duncan's multiple range test was used to verify significant differences among all treatments at $p < 0.05$. All graphs were created using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA, USA). All data are presented as the mean ± standard error (S.E.).

3. Results

3.1. Growth Characteristics in Response to Intermittent UV-B Treatment

In terms of growth parameters, no significant differences were observed in the FW and DW or the plant height of AJN plants across the different UV-B treatment periods (Figure 2). However, variations were noted in the relative water content (RWC), indicating a decrease, particularly in the UV12/C1, UV06/C2, and UV03/C4 treatments compared to the control (Table 1). The dry weights of the leaves (Figure 3A) and stems (Figure 3B) were not significantly different between the control and treatment groups; however, the leaf area decreased among all treatments compared to the control (Figure 3C). The SLA (Figure 3D) was significantly reduced in UV12/C1 and UV03/C4, with the UV12/C1 treatment being the lowest. In addition, SPAD values decreased after UV-B treatment (Figure 4).

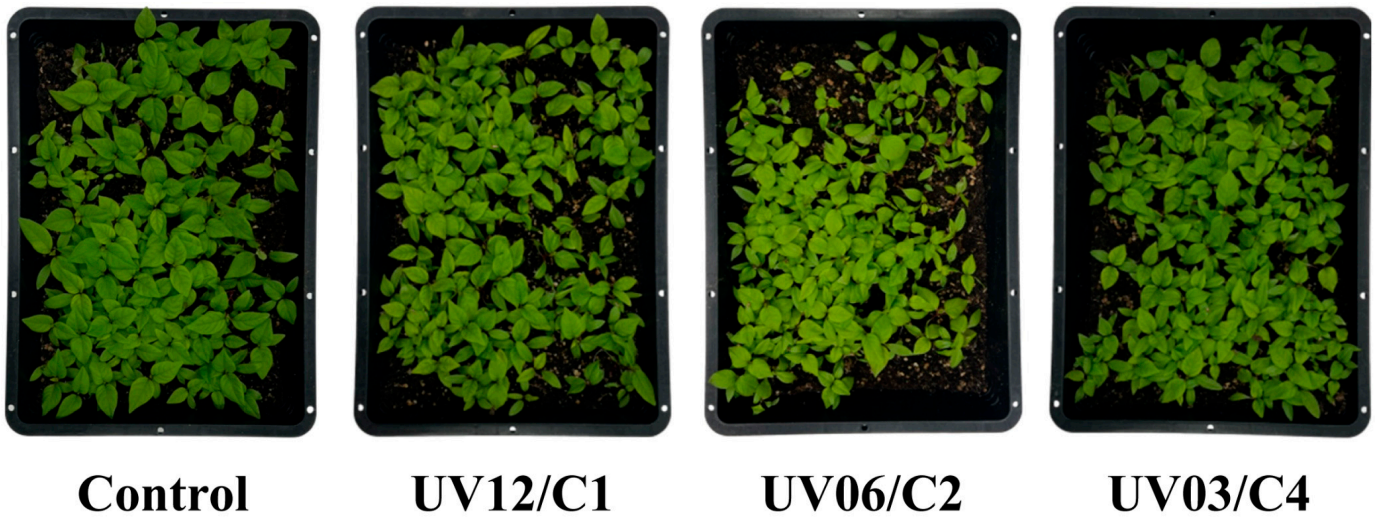

Figure 2. Growth of *Achyranthes japonica* Nakai microgreens under the different UV-B treatment periods, taken immediately after harvest, one day following the treatments.

Table 1. Growth parameters of *Achyranthes japonica* Nakai microgreens under different UV-B treatment periods.

Treatments	Growth Parameters											
	Fresh Weight (g)			Dry Weight (g)			Relative Water Content (%)			Plant Height (cm)		
Control	11.52	±	0.50 [z] a [y]	0.90	±	0.04 a	92.19	±	0.10 a	2.78	±	0.16 a
UV12/C1	10.23	±	0.48 a	0.91	±	0.03 a	91.16	±	0.22 b	2.73	±	0.13 a
UV06/C2	10.41	±	0.33 a	0.89	±	0.03 a	91.47	±	0.05 b	2.63	±	0.10 a
UV03/C4	9.93	±	0.77 a	0.85	±	0.06 a	91.53	±	0.09 b	2.68	±	0.08 a

[z] Mean ± S.E.; [y] Mean separation within column using Duncan's multiple range test at the 5% level ($n = 4$). Values in each column followed by different letters are significantly different.

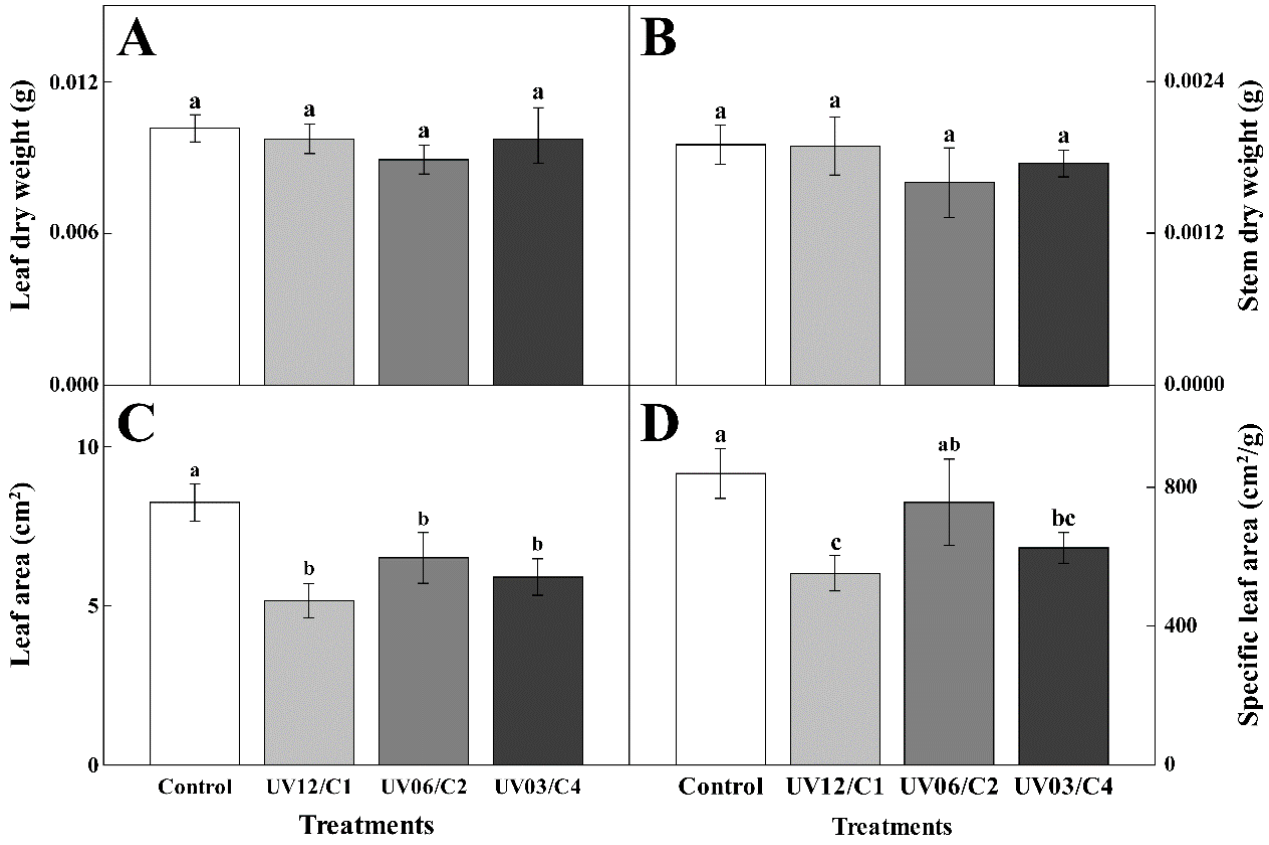

Figure 3. Leaf dry weight (**A**), stem dry weight (**B**), leaf area (**C**), and specific leaf area (**D**) of *Achranthes japonica* Nakai microgreens under the different UV-B treatment periods, with UV-B applied one day before harvest. The different letters above the bars indicate the significant differences among means ($p < 0.05$). Error bars represent the standard error of 10 replicates.

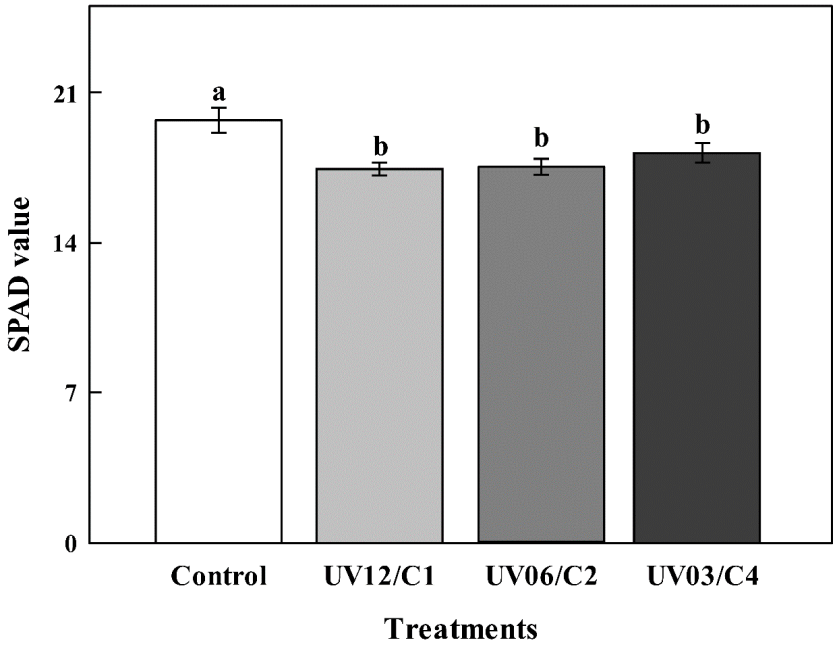

Figure 4. SPAD value of *Achyranthes japonica* Nakai microgreens under different UV-B treatment periods, with UV-B applied one day before harvest. The different letters above the bars indicate the significant differences among means ($p < 0.05$). Error bars represent the standard error of 10 replicates.

3.2. Chlorophyll Fluorescence in Response to Intermittent UV-B Treatment

The Fv/Fm values did not show a decrease in the control. In addition, in the UV12/C1 treatment, Fv/Fm decreased during the 12 h treatment but subsequently recovered. In the UV06/C2 treatment, Fv/Fm values gradually decreased with the UV-B treatment, and recovery was slower than that of the other treatments. In the UV03/C4 treatment, Fv/Fm values decreased during UV-B treatment, and recovery was fast (Figure 5).

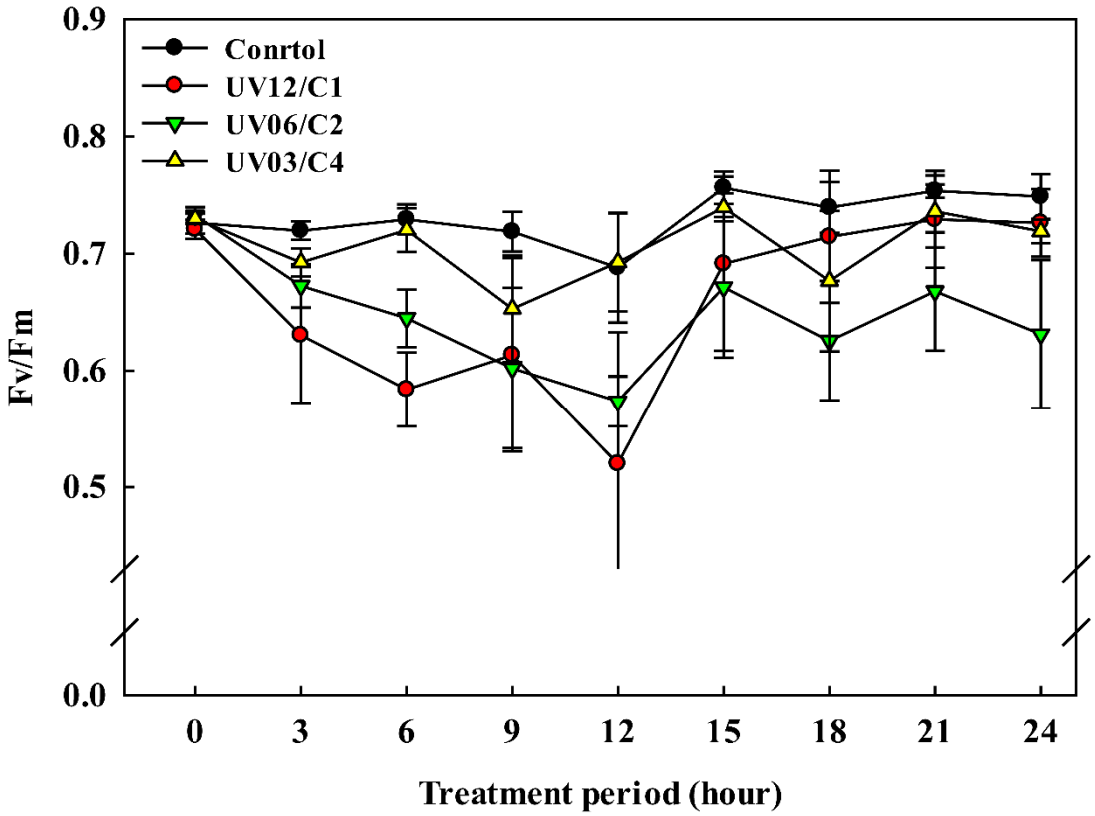

Figure 5. Variations in Fv/Fm for leaves of *Achyranthes japonica* Nakai microgreens during different UV-B treatment periods, with UV-B applied one day before harvest. Measurements were conducted once every three hours. Error bars represent the standard error of five replicates.

3.3. Bioactive Compounds, Antioxidant Properties, and 20-Hydroxyecdysone Contents in Response to Intermittent UV-B Treatment

The total phenolic (Figure 6A) and total flavonoid contents (Figure 6B) differed between the control and UV-B treatments. The total phenolic and flavonoid contents significantly increased in the UV-B treatments. The total phenolic content in all the UV-B treatments increased by about 1.5 times compared to the control, and the total flavonoid content increased by 1.4 times in UV12/C1 and UV06/C2 and 1.2 times in UV03/C4.

The AJN in this study displayed antioxidant activity by scavenging DPPH, ABTS, and FRAP (Figure 7). The DPPH and FRAP levels increased significantly in the UV12/C1 treatment (Figure 7A,C). In addition, ABTS expression increased significantly in the UV12/C1 and UV06/C2 treatments (Figure 7B).

The 20-hydroxyecdysone (20E) content differed during the UV-B treatment periods (Figure 8) but increased with all UV-B treatments. The highest rate of increase was observed in the UV06/C2 treatment, which showed 1.4-times increase compared to 1.1 times in the UV12/C1 group and 1.2 times in the UV03/C4 group. Further analysis of the relationships between bioactive compounds, antioxidant activities, and other physiological characteristics under UV-B treatments can be found in the Supplementary materials (Figure S1).

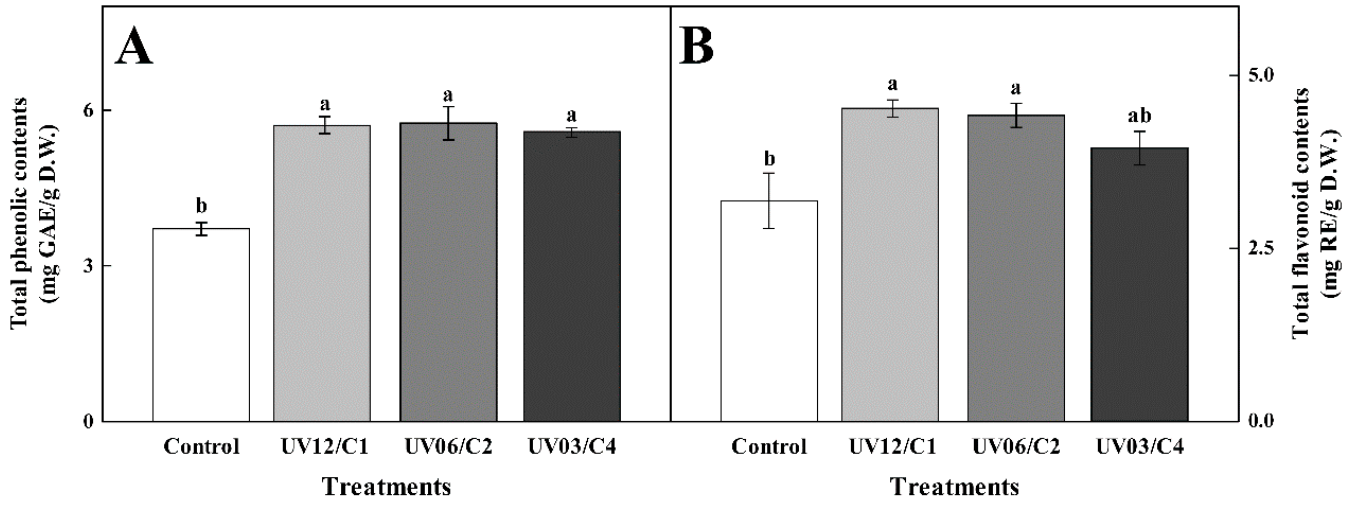

Figure 6. Total phenolic contents (**A**) and total flavonoid contents (**B**) of *Achranthes japonica* Nakai microgreens under the different UV-B treatment periods, with UV-B applied one day before harvest. The different letters above the bars indicate the significant differences among means ($p < 0.05$). Error bars represent the standard error of four replicates.

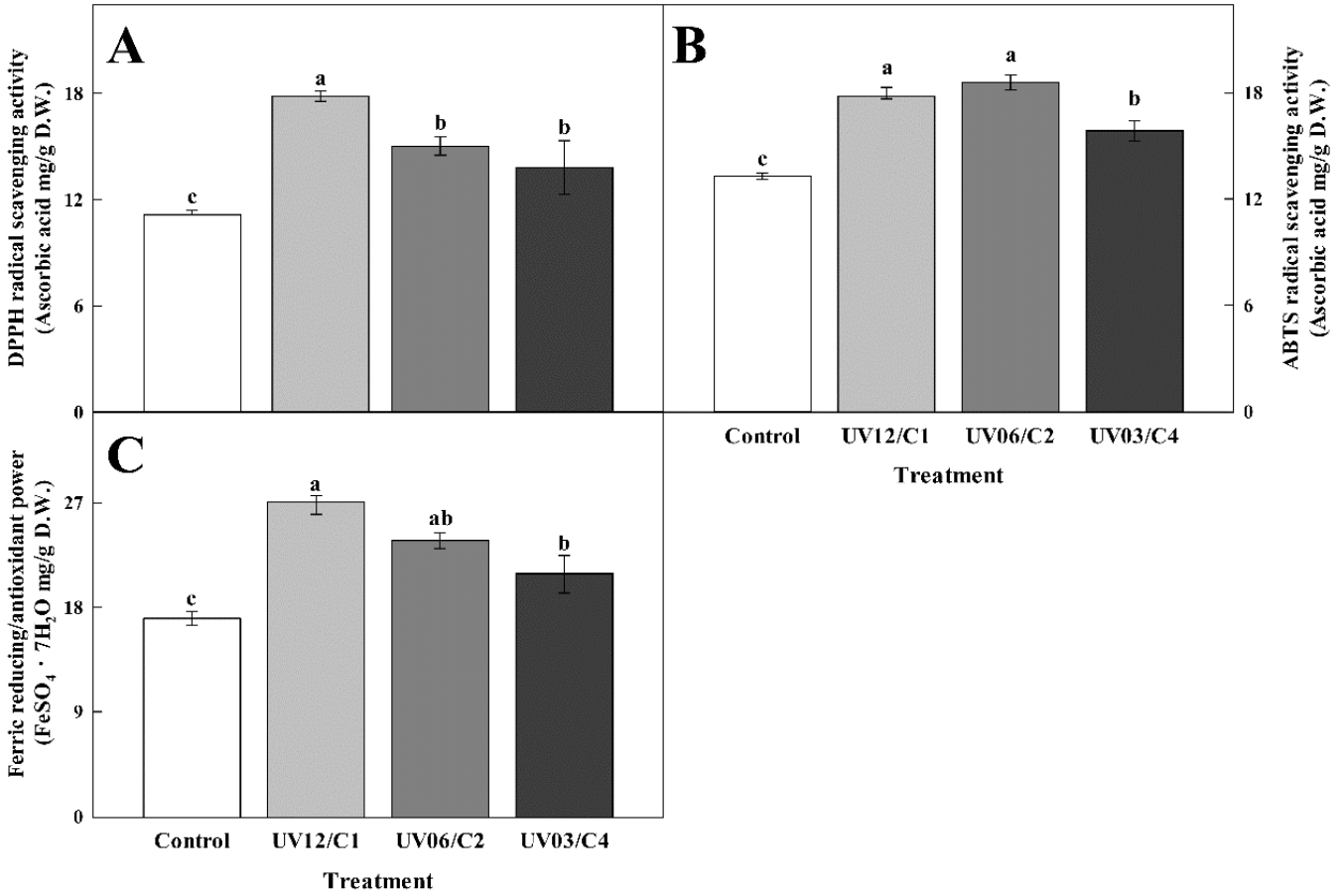

Figure 7. Antioxidant activities of DPPH (**A**), ABTS (**B**), and FRAP (**C**) of *Achranthes japonica* Nakai microgreens under the different UV-B treatment periods, with UV-B applied one day before harvest. The different letters above the bars indicate the significant differences among means ($p < 0.05$). Error bars represent the standard error of four replicates.

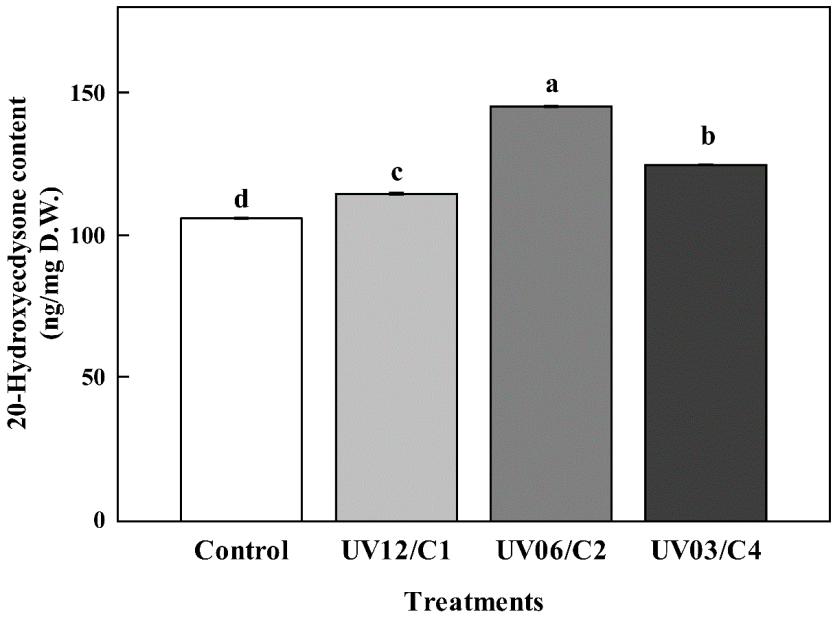

Figure 8. 20-Hydroxyecdysone content of *Achyranthes japonica* Nakai microgreens under the different UV-B treatment periods, with UV-B applied one day before harvest. The different letters above the bars indicate the significant differences among means ($p < 0.05$). Error bars represent the standard error of three replicates.

4. Discussion

4.1. Growth Characteristics

Biomass is a reliable indicator of plant sensitivity to UV-B as represents the cumulative effect of disrupted or suppressed physiological functions. Various studies have demonstrated that UV-B treatment has many effects on plants, including the inhibition of photosynthesis [36], reduced growth [37], and abnormal morphogenesis [38]. However, in this study, there were no significant differences in DW, FW, or plant length between the UV-B treatments and control (Figure 3). These results are consistent with those of UV-B irradiation of broccoli sprouts [39] for 24 h before harvest and the finding that short-term UV-B irradiation of amaranth leaves [26] did not affect biomass. This is because short-term treatment with UV-B has no negative effects and does not cause abnormal external morphology [26]. Reducing RWC is an essential adaptive plant strategy for reducing environmental stress. Compared with the control, the decrease in RWC under UV-B irradiation was consistent with the results for *Hippophae rhamnoides* [40] and soybean leaves [41].

The finding that UV-B treatment reduced SLA is consistent with that of other studies [32]. SLA reduction is a morphological strategy of UV-B-stressed plants indicating an increase in leaf thickness or mass density. Therefore, these results indicate that UV treatment resulted in thicker and more compact leaves.

A decrease in leaf area occurs owing to a reduction in the rate of photosynthesis, which has a cumulative effect on impaired or suppressed physiological functions [42]. A reduction in leaf area is also a helpful indicator of the potential sensitivity of plants to UV-B radiation [43]. Hence, the results of this study imply that AJN is influenced by UV-B stress. Plant morphology serves as a sensitive marker for UV-B damage, and responses to UV-B may significantly differ among plant species, which underscores the nuanced nature of plant reactions to UV-B exposure. In other studies, UV-B dosage did not have as strong a negative impact on basil growth but instead induced active physiological responses in plants [44]. Building upon this, in this study, while there were no significant differences in biomass, plant height, or plant weight, leaf area slightly decreased with UV-B treatment. These results suggest that plants exhibit a sensitive response in morphological changes when exposed to UV-B. However, overall, UV-B radiation did not exert a significant

negative impact on overall plant growth. Therefore, this indicates that plant responses to UV-B vary depending on the species, and that active physiological responses may be induced depending on the species.

SPAD values are an indicator of leaf greening or biological chlorophyll content and are used as an indicator of plant health [45]. The results of this study are similar to a previous study in which SPAD values decreased with UV exposure [46] (Figure 4). In a study on *Peucedanum japonicum*, SPAD values decreased as SLA values decreased [47]. This finding is consistent with the results of this study. The SPAD index and UV rays can indirectly affect the photosynthetic process through the photodegradation of photosynthetic pigments [48,49]. Moreover, SPAD values may increase with leaf thickness and specific leaf mass [50] and exhibit a highly positive correlation with chlorophyll content (a + b) [51]. In line with these observations, a study by Yao et al. [52] highlighted that intensified UV-B radiation can suppress the production of photosynthetic pigments and disrupt the structure and membrane systems of chloroplasts. This underscores the intricate relationship between UV radiation and the photosynthetic process, emphasizing the importance of understanding such interactions for elucidating plant responses to environmental stressors.

4.2. Chlorophyll Fluorescence

The Fv/Fm ratio is commonly used as an indicator of stress caused by photoinhibition [53] and the potential quantum shock (photochemical efficiency) of PSII [54]. Fv/Fm ratio readings greater than 0.8 indicate optimal light usage efficiency, whereas values between 0.7 and 0.8 indicate that the plant is unhindered in growth and photosystem II efficiency is optimal [55]. The decrease in the Fv/Fm ratio due to UV-B irradiation in the present study was consistent with previous results for soybeans [49] (Figure 5). These results imply that the plants were stressed by UV-B. This was caused by reduced photosynthetic capacity owing to PSII-related protein degradation [56]. The Fv/Fm values of the UV12/C1 treatment almost recovered to control levels during the dark period, and it was observed from the results of the UV03/C4 treatment that 3 h is sufficient for recovery to control levels. Bischof et al. [57] also showed that Fv/Fm values recovered after a certain period of UV-B irradiation. Specifically, when cells were treated with UV-B for 12 h in the presence of light, their ability to recover was better than that when cells were treated for 6 h and 3 h. This is because the cells of plants are more sensitive to UV-B stress during the night than during the day, and light is required to protect them against UV-B [58]. Plants are more resistant to UV-B radiation under constant light than under dark conditions [58] as protective pigment biosynthesis is suppressed under dark conditions [59]. Fv/Fm recovery was better under the UV03/C4 treatment compared to that under the UV06/C2 treatment. This may have occurred through various mechanisms involving continuous and intermittent stress. Adaptation to intermittent stress involves a priming effect caused by exposure to mildly stressful stimuli. Consequently, tolerance to more severe stress can be achieved [60]. With defense priming, plants respond to subsequent stress situations faster and with more robust defense responses [61].

4.3. Bioactive Compounds

One of the mechanisms by which plants adapt to UV-B stress is in their ability to increase the production of secondary metabolites in leaf tissues [62]. In this experiment, even 12 h of UV-B treatment right before harvest, at a specific intensity and wavelength, was sufficient to induce UV-B stress in AJN microgreens, leading to an increase in bioactive compounds. Likewise, previous research has also reported that short-term UV-B irradiation can increase the levels of bioactive compounds, such as phenolic compounds and flavonoids, in plants [63–65]. These UV-B-inducing compounds protect plants from UV damage, showcasing a plant's response to environmental stressors, as critical enzymes in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase, are strongly stimulated by various environmental stressors [40]. Notably, flavonoids, a major class of phenolic compounds, are synthesized in response to UV-B exposure. Flavonoids possess radical-

scavenging abilities and play a crucial role in photoprotection against UV-B radiation [66]. Additionally, they contribute to maintaining optimal levels of photosynthetic pigments and regular photosynthetic activity [67]. This adaptive mechanism is well documented, indicating a coordinated response of plants to UV-B stressors [68]. Studies have highlighted that UV-B-mediated flavonoid accumulation not only aids in removing ROS but also serves as a shield against UV radiation, underscoring its significance in plant defense mechanisms [68].

Although all the UV-B treatments showed higher levels of total phenolic and total flavonoid contents compared to the control, these results represent the total contents rather than the levels of individual compounds (Figure 6). Therefore, there are limitations in assessing trends in compound levels across different recovery times.

4.4. Antioxidant Properties

Antioxidant properties are used to determine the antioxidant capacities of fruits [69] and vegetables [70]. DPPH is used as an indicator for radical-scavenging anion activity [71], ABTS is used as an indicator of radical-scavenging cation activity [72], and FRAP catalyzes the transfer of hydrogen ions to Fe^{3+} to stabilize the radical and reduce it to Fe^{2+} [73]. The results of this study are consistent with the finding that UV-B irradiation increases the DPPH radical-scavenging activity in buckwheat sprouts [74]. Antioxidant capacity (DPPH, ABTS, and FRAP) is directly related to an increase in phenolic compounds [75]. Phenolic compounds participate in the antioxidant system [76], and their increase can lead to an increase in antioxidant capacity [77]. Therefore, the increase in antioxidant capacity due to UV-B irradiation was due to phenolic compounds. However, the antioxidant capacity decreased in response to UV-B irradiation during the dark period compared to irradiation during the light period. These results were consistent with those reported for *Perilla frutescens* [29], in which no new photo-assimilates were produced during the dark period. Therefore, generating the resources necessary to synthesize antioxidant enzymes and bioactive compounds to prevent the UV-mediated overproduction of ROS is difficult [78].

4.5. 20-Hydroxyecdysone Content

Numerous studies have demonstrated that UV light stimulates the synthesis of secondary metabolites, including terpenoids, phenols, flavonoids, and steroids in tropical medicinal plant species [79]. Belonging to the steroid family, 20E is the major phytoecdysteroid present in plants [80]. In addition, 20E has potential for biotechnological, pharmacological, medicinal, and agrochemical applications [81]. UV-B induces the production of secondary metabolites in plants; however, its effect on 20-hydroxyecdysone remains unclear. When the two varieties of Brazilian ginseng plants were irradiated with UV-B, 20E significantly increased in the leaves and roots; when they were irradiated for 2 h, it decreased in the stems; and when they were irradiated for 2 h, it increased. In comparison, other cultivars did not display significant changes in 20E content. ROS caused by UV-B promote organized signaling, leading to the upregulation and increased content of the 20E gene [81]. This shows that the effect of 20E varies depending on the UV-B irradiation time and plant species. The increase in 20E after UV-B treatment is due to the accumulation of phytosterols and triterpene compounds, which reduce the damage caused by UV-B radiation [79]. In addition, the increase and decrease of specific metabolites varies depending on physiological age, UV-B exposure time, UV-B dose, and other environmental parameters [82]. Therefore, considering these conditions, it is necessary to develop cultivation strategies for enhancing crop quality through UV-B exposure.

Under the UV06/C2 treatment, DPPH and FRAP decreased compared with the UV12/C1 treatment; however, 20E increased (Figures 7 and 8). This is consistent with results showing that 20E has weak radical-scavenging activity toward DPPH [83,84]. The antioxidant effect was due to the co-elution of other phenolic compounds with 20E rather than with 20E alone. This is because ecdysteoid molecules do not have a structure responsible for the antioxidant effects commonly found in most known antioxidants [85]. These results demonstrate the weak radical-scavenging activity of 20E against DPPH and FRAP.

In relation to this, we suggest that the antioxidant effect of 20E becomes more pronounced when it is used in conjunction with other phenolic compounds.

5. Conclusions

In this study, the effects of UV-B irradiation on the growth and bioactive compounds of pre-harvest AJN microgreens were demonstrated in a vertical farm. UV-B treatment did not significantly alter fresh weight (FW), dry weight (DW), or plant height but led to a reduction in relative water content (RWC), leaf area, specific leaf area (SLA), and chlorophyll content (SPAD). The Fv/Fm ratio did not decrease in the control but decreased during UV-B treatment in the UV12/C1 treatment, with values returning to a healthy state through a 12 h recovery period. In the UV06/02 treatment, Fv/Fm recovery was slower than that in the UV03/C4 treatment. The UV-B treatment significantly increased total phenol and flavonoid contents as well as antioxidant capacity, with a notable increase in 20E content, particularly with the UV06/C2 treatment.

This study confirms that the UV-B treatment of AJN microgreens before harvesting increases phenol, flavonoid, and antioxidant contents, particularly 20E content. Therefore, the UV06/C2 treatment is judged to be effective for increasing 20E content, and appropriate UV-B irradiation with a recovery period and intermittent treatment could improve the nutritional quality of AJN microgreens in vertical farms. Optimizing these methods can improve the health benefits and market value AJN microgreens, highlighting their potential for broader use in the food and agricultural sectors.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10101040/s1, Figure S1; Normalized heatmap (A) and Pearson correlation matrix (B) average growth parameters and physiological characteristics in *Achyranthes japonica* Nakai microgreens for the UV-B treatment period.

Author Contributions: K.-H.S. conceived the project; H.-S.S., G.O.L., I.-J.K. and G.-S.Y. performed the experiments; Y.L.K. wrote the manuscript; M.-S.Y. and J.G.Y. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through the Technology Commercialization Support Program, funded by the Ministry of Agriculture, Food and Rural Affairs (MAFRA) (821037031HD020), and a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (no. 2021R1F1A1047454).

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: We are grateful for helpful co-working and discussions with the members of Yun's laboratory.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Um, M.S. The evaluation of Anti-oxidation, Anti-inflammation and Anti-wrinkle activity of Jeju Native *Achyranthes japonica* Nakai. *J. Korean Appl. Sci. Technol.* **2021**, *38*, 1209–1218. [CrossRef]
2. Seo, S.J.; Kim, N.W. Antioxidant activities of extracts from leaves and stems of *Achyranthes japonica*. *J. Korean Soc. Food Sci. Nutr.* **2014**, *43*, 972–979. [CrossRef]
3. Boo, K.H.; Lee, D.; Van Nguyen, Q.; Jin, S.B.; Kang, S.; Viet, C.D.; Park, S.P.; Lee, D.S.; Riu, K.Z. Fluctuation of 20-hydroxyecdysone in individual organs of *Achyranthes japonica* during reproductive growth stage and its accumulation into seed. *J. Korean Soc. Appl. Biol. Chem.* **2013**, *56*, 335–338. [CrossRef]
4. Lee, C.W.; Lee, D.Y. Metabolomic Investigation on Fermentation Products of *Achyranthes japonica* Nakai by *Lactobacillus plantarum*. *J. Microbiol. Biotechnol.* **2020**, *30*, 378–381. [CrossRef] [PubMed]
5. Orie, N.N.; Raees, A.; Aljaber, M.Y.; Mohamed-Ali, N.; Bensmail, H.; Hamza, M.M.; Al-Ansari, N.; Beotra, A.; Mohamed-Ali, V.; Almaadheed, M. 20-Hydroxyecdysone dilates muscle arterioles in a nitric oxide-dependent, estrogen ER-β receptor-independent manner. *Phytomed. Plus* **2021**, *1*, 100078. [CrossRef]

6. Harakotr, B.; Srijunteuk, S.; Rithichai, P.; Tabunhan, S. Effects of light-emitting diode light irradiance levels on yield, antioxidants and antioxidant capacities of indigenous vegetable microgreens. *Sci. Technol. Asia* **2019**, *24*, 59–66. [CrossRef]
7. Goto, E. Production of pharmaceuticals in a specially designed plant factory. In *Plant Factory*; Kozai, T., Niu, G., Takagaki, M., Eds.; Academic Press: Cambridge, MA, USA, 2016; Volume 15, pp. 193–200. [CrossRef]
8. Erekath, S.; Seidlitz, H.; Schreiner, M.; Dreyer, C. Food for future: Exploring cutting-edge technology and practices in vertical farm. *Sustain. Cities Soc.* **2024**, *106*, 105357. [CrossRef]
9. Wicharuck, S.; Khongdee, N.; Pripanakul, T.; Whangchai, N.; Pimpimol, T.; Chaichana, C. Vertical farming: A potential farming practice for lettuce production. *Chil. J. Agric. Res.* **2023**, *83*, 248–259. [CrossRef]
10. Halgamuge, M.N.; Bojovschi, A.; Fisher, P.M.; Le, T.C.; Adeloju, S.; Murphy, S. Internet of Things and autonomous control for vertical cultivation walls towards smart food growing: A review. *Urban For. Urban Green.* **2021**, *61*, 127094. [CrossRef]
11. Zeljković, S.Ć.; Aucique-Perez, C.E.; Štefelová, N.; De Diego, N. Optimizing growing conditions for hydroponic farming of selected medicinal and aromatic plants. *Food Chem.* **2022**, *375*, 131845. [CrossRef] [PubMed]
12. Bafort, F.; Kohnen, S.; Maron, E.; Bouhadada, A.; Ancion, N.; Crutzen, N.; Jijakli, M.H. The agro-economic feasibility of growing the medicinal plant *Euphorbia peplus* in a modified vertical hydroponic shipping container. *Horticulture* **2022**, *8*, 256. [CrossRef]
13. Xiao, Z.; Lester, G.E.; Luo, Y.; Wang, Q. Assessment of vitamin and carotenoid concentrations of emerging food products: Edible microgreens. *J. Agric. Food Chem.* **2012**, *60*, 7644–7651. [CrossRef]
14. Sarıkamış, G.; Yıldırım, A.; Alkan, D. Glucosinolates in seeds, sprouts and seedlings of cabbage and black radish as sources of bioactive compounds. *Can. J. Plant Sci.* **2015**, *95*, 681–687. [CrossRef]
15. Graamans, L.; Baeza, E.; Van Den Dobbelsteen, A.; Tsafaras, I.; Stanghellini, C. Plant factories versus greenhouses: Comparison of resource use efficiency. *Agric. Syst.* **2018**, *160*, 31–43. [CrossRef]
16. Klem, K.; Gargallo-Garriga, A.; Rattanapichai, W.; Oravec, M.; Holub, P.; Veselá, B.; Sardans, J.; Penuelas, J.; Urban, O. Distinct morphological, physiological, and biochemical responses to light quality in barley leaves and roots. *Front. Plant Sci.* **2019**, *10*, 1026. [CrossRef]
17. Xue, A.; Liang, W.; Wen, S.; Gao, Y.; Huang, X.; Tong, Y.; Hao, Y.; Luo, L. Metabolomic analysis based on EESI-MS indicate blue LED light promotes aliphatic-glucosinolates biosynthesis in broccoli sprouts. *J. Food Compos. Anal.* **2021**, *97*, 103777. [CrossRef]
18. Kopsell, D.A.; Sams, C.E. Increases in shoot tissue pigments, glucosinolates, and mineral elements in sprouting broccoli after exposure to short-duration blue light from light emitting diodes. *J. Am. Soc. Hortic. Sci.* **2013**, *138*, 31–37. [CrossRef]
19. Brazaityte, A.; Virsile, A.; Jankauskiene, J.; Sakalauskiene, S.; Samuoliene, G.; Sirtautas, R.; Novickovas, A.; Dabasinskas, L.; Miliauskiene, J.; Vastakaite, V.; et al. Effect of supplemental UV-A irradiation in solid-state lighting on the growth and phytochemical content of microgreens. *Int. Agrophys.* **2015**, *29*, 13–22. [CrossRef]
20. Caracciolo, F.; El-Nakhel, C.; Raimondo, M.; Kyriacou, M.C.; Cembalo, L.; De Pascale, S.; Rouphael, Y. Sensory attributes and consumer acceptability of 12 microgreens species. *Agronomy* **2020**, *10*, 1043. [CrossRef]
21. Demir, K.; Sarıkamış, G.; Seyrek, G.Ç. Effect of LED lights on the growth, nutritional quality and glucosinolate content of broccoli, cabbage and radish microgreens. *Food Chem.* **2023**, *401*, 134088. [CrossRef]
22. Santin, M.; Sciampagna, M.C.; Mannucci, A.; Puccinelli, M.; Angelini, L.G.; Tavarini, S.; Accorsi, M.; Incrocci, L.; Ranieri, A.; Castagna, A. Supplemental UV-B exposure influences the biomass and the content of bioactive compounds in *Linum usitatissimum* L. sprouts and microgreens. *Horticulture* **2022**, *8*, 213. [CrossRef]
23. Kuhlmann, F.; Müller, C. Independent responses to ultraviolet radiation and herbivore attack in broccoli. *J. Exp. Bot.* **2009**, *60*, 3467–3475. [CrossRef]
24. Singh, P.; Singh, A.; Choudhary, K.K. Revisiting the role of phenylpropanoids in plant defense against UV-B stress. *Plant Stress* **2023**, *7*, 100143. [CrossRef]
25. Inostroza-Blancheteau, C.; Acevedo, P.; Loyola, R.; Arce-Johnson, P.; Alberdi, M.; Reyes-Díaz, M. Short-term UV-B radiation affects photosynthetic performance and antioxidant gene expression in highbush blueberry leaves. *Plant Physiol. Biochem.* **2016**, *107*, 301–309. [CrossRef]
26. Wittayathanarattana, T.; Wanichananan, P.; Supaibulwatana, K.; Goto, E. Enhancement of bioactive compounds in baby leaf *Amaranthus tricolor* L. using short-term application of UV-B irradiation. *Plant Physiol. Biochem.* **2022**, *182*, 202–215. [CrossRef] [PubMed]
27. Jang, S.N.; Lee, G.O.; Sim, H.S.; Bae, J.S.; Lee, A.R.; Cho, D.Y.; Cho, K.M.; Son, K.H. Effect of Pre-Harvest Irradiation of UV-A and UV-B LED in Ginsenosides Content of Ginseng Sprouts. *J. Bio-Environ. Control* **2022**, *31*, 28–34. [CrossRef]
28. Yang, W.; Qian, G.; Chen, Y.; Liu, T.; Wan, H.; Wang, S.; Meng, X.; Chen, W.; Atia-tul-Wahab; Su, Y.; et al. Profiling of polyphenols for in-depth understanding of Tartary buckwheat sprouts: Correlation between cultivars and active components, dynamic changes and the effects of ultraviolet B stress. *Food Chem. X* **2022**, *14*, 100295. [CrossRef]
29. Yoshida, H.; Shimada, K.; Hikosaka, S.; Goto, E. Effect of UV-B irradiation on bioactive compounds of red perilla (*Perilla frutescens* (L.) britton) cultivated in a plant factory with artificial light. *Horticulture* **2022**, *8*, 725. [CrossRef]
30. Oyebanji, O.B.; Nweke, O.; Odebunmi, O.; Galadima, N.B.; Idris, M.S.; Nnodi, U.N.; Afolabi, A.S.; Ogbadu, G.H. Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. *Afr. J. Biotechnol.* **2009**, *8*, 5395–5399.
31. Garnier, E.; Laurent, G. Leaf anatomy, specific mass and water content in congeneric annual and perennial grass species. *New Phytol.* **1994**, *128*, 725–736. [CrossRef]

32. Cuzzuol, G.R.F.; Gama, V.N.; Zanetti, L.V.; Werner, E.T.; Pezzopane, J.E.M. UV-B effects on growth, photosynthesis, total antioxidant potential and cell wall components of shade-tolerant and sun-tolerant ecotypes of *Paubrasilia echinata*. *Flora* **2020**, *271*, 151679. [CrossRef]
33. Ainsworth, E.A.; Gillespie, K.M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat. Protoc.* **2007**, *2*, 875–877. [CrossRef] [PubMed]
34. Lee, J.H.; Kim, S.C.; Lee, H.Y.; Cho, D.Y.; Jung, J.G.; Kang, D.; Kang, S.S.; Cho, K.M. Changes in nutritional compositions of processed mountain-cultivated ginseng sprouts (*Panax ginseng*) and screening for their antioxidant and anti-inflammatory properties. *J. Funct. Food.* **2021**, *86*, 104668. [CrossRef]
35. Lee, H.Y.; Cho, D.Y.; Jang, K.J.; Lee, J.H.; Jung, J.G.; Kim, M.J.; Jeong, J.B.; Haque, M.A.; Cho, K.M. Changes of γ-aminobutyric acid, phytoestrogens, and biofunctional properties of the isoflavone-enriched soybean (*Glycine max*) leaves during solid lactic acid fermentation. *Fermentation* **2022**, *8*, 525. [CrossRef]
36. Kataria, S.; Jajoo, A.; Guruprasad, K.N. Impact of increasing Ultraviolet-B (UV-B) radiation on photosynthetic processes. *J. Photochem. Photobiol. B Biol.* **2014**, *137*, 55–66. [CrossRef]
37. Fina, J.; Casadevall, R.; AbdElgawad, H.; Prinsen, E.; Markakis, M.N.; Beemster, G.T.; Casati, P. UV-B inhibits leaf growth through changes in growth regulating factors and gibberellin levels. *Plant Physiol.* **2017**, *174*, 1110–1126. [CrossRef]
38. Chen, M.; Huang, Y.; Liu, G.; Qin, F.; Yang, S.; Xu, X. Effects of enhanced UV-B radiation on morphology, physiology, biomass, leaf anatomy and ultrastructure in male and female mulberry (*Morus alba*) saplings. *Environ. Exp. Bot.* **2016**, *129*, 85–93. [CrossRef]
39. Moreira-Rodríguez, M.; Nair, V.; Benavides, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. UVA, UVB light doses and harvesting time differentially tailor glucosinolate and phenolic profiles in broccoli sprouts. *Molecules* **2017**, *22*, 1065. [CrossRef] [PubMed]
40. Yang, Y.; Yao, Y.; He, H. Influence of ambient and enhanced ultraviolet-B radiation on the plant growth and physiological properties in two contrasting populations of *Hippophae rhamnoides*. *J. Plant Res.* **2008**, *121*, 377–385. [CrossRef]
41. Zhao, M.G.; Liu, Y.G.; Zhang, L.X.; Zheng, L.; Bi, Y.R. Effects of enhanced UV-B radiation on the activity and expression of alternative oxidase in red kidney bean leaves. *J. Integr. Plant Biol.* **2007**, *49*, 1320–1326. [CrossRef]
42. González, J.A.; Rosa, M.; Parrado, M.F.; Hilal, M.; Prado, F.E. Morphological and physiological responses of two varieties of a highland species (*Chenopodium quinoa* Willd.) growing under near-ambient and strongly reduced solar UV–B in a lowland location. *J. Photochem. Photobiol. B Biol.* **2009**, *96*, 144–151. [CrossRef] [PubMed]
43. Wand, S.J. Concentration of ultraviolet-B radiation absorbing compounds in leaves of a range of fynbos species. *Vegetatio* **1995**, *116*, 51–61. [CrossRef]
44. Sakalauskaitė, J.; Viskelis, P.; Dambrauskienė, E.; Sakalauskienė, S.; Samuolienė, G.; Brazaitytė, A.; Duchovskis, P.; Urbonavičienė, D. The effects of different UV-B radiation intensities on morphological and biochemical characteristics in *Ocimum basilicum* L. *J. Sci. Food Agric.* **2013**, *93*, 1266–1271. [CrossRef]
45. Netto, A.T.; Campostrini, E.; de Oliveira, J.G.; Bressan-Smith, R.E. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. *Sci. Hortic.* **2005**, *104*, 199–209. [CrossRef]
46. Kim, H.M.; Hwang, S.J. The growth and development of 'mini chal' tomato plug seedlings grown under various wavelengths using light emitting diodes. *Agronomy* **2019**, *9*, 157. [CrossRef]
47. Song, K.S.; Jeon, K.S.; Choi, K.S.; Kim, C.H.; Park, Y.B.; Kim, J.J. Characteristics of photosynthesis and leaf growth of *Peucedanum japonicum* by leaf mold and shading level in forest farming. *Korean J. Med. Crop Sci.* **2015**, *23*, 43–48. [CrossRef]
48. Jansen, M.A.; Hectors, K.; O'Brien, N.M.; Guisez, Y.; Potters, G. Plant stress and human health: Do human consumers benefit from UV-B acclimated crops? *Plant Sci.* **2008**, *175*, 449–458. [CrossRef]
49. Li, X.; Zhang, L.; Li, Y.; Ma, L.; Bu, N.; Ma, C. Changes in photosynthesis, antioxidant enzymes and lipid peroxidation in soybean seedlings exposed to UV-B radiation and/or Cd. *Plant Soil* **2012**, *352*, 377–387. [CrossRef]
50. Marenco, R.A.; Antezana-Vera, S.A.; Nascimento, H.C.S. Relationship between specific leaf area, leaf thickness, leaf water content and SPAD-502 readings in six Amazonian tree species. *Photosynthetica* **2009**, *47*, 184–190. [CrossRef]
51. Mielke, M.S.; Schaffer, B.; Li, C. Use of a SPAD meter to estimate chlorophyll content in *Eugenia uniflora* L. leaves as affected by contrasting light environments and soil flooding. *Photosynthetica* **2010**, *48*, 332–338. [CrossRef]
52. Yao, X.; Liu, Q. Changes in morphological, photosynthetic and physiological responses of Mono Maple seedlings to enhanced UV-B and to nitrogen addition. *Plant Growth Regul.* **2006**, *50*, 165–177. [CrossRef]
53. Bjerke, J.W.; Gwynn-Jones, D.; Callaghan, T.V. Effects of enhanced UV-B radiation in the field on the concentration of phenolics and chlorophyll fluorescence in two boreal and arctic–alpine lichens. *Environ. Exp. Bot.* **2005**, *53*, 139–149. [CrossRef]
54. Genty, B.; Briantais, J.M.; Baker, N.R. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biocim. Biophys. Acta Gen. Subj.* **1989**, *990*, 87–92. [CrossRef]
55. Yobo, K.S.; Laing, M.D.; Hunter, C.H. Effects of single and dual applications of selected Trichoderma and Bacillus isolates on performance of dry bean seedlings grown in composted pine bark growth medium under shadehouse conditions. *J. Plant Nutr.* **2009**, *32*, 1271–1289. [CrossRef]
56. Ohad, I.; Kyle, D.J.; Arntzen, C.J. Membrane protein damage and repair: Removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes. *J. Cell Biol.* **1984**, *99*, 481–485. [CrossRef] [PubMed]
57. Bischof, K.; Hanelt, D.; Wiencke, C. UV-radiation can affect depth-zonation of Antarctic macroalgae. *Mar. Biol.* **1998**, *131*, 597–605. [CrossRef]

58. Takeuchi, T.; Newton, L.; Burkhardt, A.; Mason, S.; Farré, E.M. Light and the circadian clock mediate time-specific changes in sensitivity to UV-B stress under light/dark cycles. *J. Exp. Bot.* **2014**, *65*, 6003–6012. [CrossRef] [PubMed]
59. Chalker-Scott, L. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* **1999**, *70*, 1–9. [CrossRef]
60. Charng, Y.Y.; Mitra, S.; Yu, S.J. Maintenance of abiotic stress memory in plants: Lessons learned from heat acclimation. *Plant Cell* **2023**, *35*, 187–200. [CrossRef] [PubMed]
61. Conrath, U.; Beckers, G.J.; Langenbach, C.J.; Jaskiewicz, M.R. Priming for enhanced defense. *Annu. Rev. Phytopathol.* **2015**, *53*, 97–119. [CrossRef] [PubMed]
62. Kakani, V.G.; Reddy, K.R.; Zhao, D.; Sailaja, K. Field crop responses to ultraviolet-B radiation: A review. *Agric. For. Meteorol.* **2003**, *120*, 191–218. [CrossRef]
63. Chen, Y.; Zhang, X.; Guo, Q.; Liu, L.; Li, C.; Cao, L.; Qin, Q.; Zhao, M.; Wang, W. Effects of UV-B radiation on the content of bioactive components and the antioxidant activity of *Prunella vulgaris* L. Spica during development. *Molecules* **2018**, *23*, 989. [CrossRef] [PubMed]
64. Jordan, B.R. Molecular response of plant cells to UV-B stress. *Funct. Plant Biol.* **2002**, *29*, 909–916. [CrossRef]
65. Li, W.; Tan, L.; Zou, Y.; Tan, X.; Huang, J.; Chen, W.; Tang, Q. The effects of ultraviolet A/B treatments on anthocyanin accumulation and gene expression in dark-purple tea cultivar 'Ziyan' (*Camellia sinensis*). *Molecules* **2020**, *25*, 354. [CrossRef]
66. Bors, W.; Heller, W.; Michael, M. The chemistry of flavonoids. In *Flavonoids in Health and Disease*; Rice-Evans, C.A., Packer, L., Eds.; Marcel Dekker: New York, NY, USA, 1998; Volume 1, pp. 111–136.
67. Day, T.A.; Neale, P.J. Effects of UV-B radiation on terrestrial and aquatic primary producers. *Annu. Rev. Ecol. Evol. Syst.* **2002**, *33*, 371–396. [CrossRef]
68. Schreiner, M.; Martínez-Abaigar, J.; Glaab, J.; Jansen, M. UV-B induced secondary plant metabolites: Potential benefits for plant and human health. *Opt. Photonik* **2014**, *9*, 34–37. [CrossRef]
69. Hashemi, S.M.B.; Khaneghah, A.M.; Barba, F.J.; Nemati, Z.; Shokofti, S.S.; Alizadeh, F. Fermented sweet lemon juice (*Citrus limetta*) using *Lactobacillus plantarum* LS5: Chemical composition, antioxidant and antibacterial activities. *J. Funct. Foods* **2017**, *38*, 409–414. [CrossRef]
70. Cho, K.M.; Ha, T.J.; Lee, Y.B.; Seo, W.D.; Kim, J.Y.; Ryu, H.W.; Jeong, H.S.; Kang, M.K.; Lee, J.H. Soluble phenolics and antioxidant properties of soybean (*Glycine max* L.) cultivars with varying seed coat colours. *J. Funct. Foods* **2013**, *5*, 1065–1076. [CrossRef]
71. Lee, S.O.; Kim, M.J.; Kim, D.G.; Choi, H.J. Antioxidative activities of temperature-stepwise water extracts from *Inonotus obliquus*. *J. Korean Soc. Food Sci. Nutr.* **2005**, *34*, 139–147. [CrossRef]
72. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
73. Park, S.J. Antioxidant activities and whitening effects of ethanol extract from Panax ginseng sprout powder. *J. Korean Soc. Food Sci. Nutr.* **2019**, *48*, 276–281. [CrossRef]
74. Tsurunaga, Y.; Takahashi, T.; Katsube, T.; Kudo, A.; Kuramitsu, O.; Ishiwata, M.; Matsumoto, S. Effects of UV-B irradiation on the levels of anthocyanin, rutin and radical scavenging activity of buckwheat sprouts. *Food Chem.* **2013**, *141*, 552–556. [CrossRef]
75. Martínez-Zamora, L.; Castillejo, N.; Artés-Hernández, F. UV-B radiation as abiotic elicitor to enhance phytochemicals and development of red cabbage sprouts. *Horticulture* **2021**, *7*, 567. [CrossRef]
76. Sakihama, Y.; Cohen, M.F.; Grace, S.C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. *Toxicology* **2002**, *177*, 67–80. [CrossRef]
77. Puccinelli, M.; Maggini, R.; Angelini, L.G.; Santin, M.; Landi, M.; Tavarini, S.; Castagna, A.; Incrocci, L. Can light spectrum composition increase growth and nutritional quality of *Linum usitatissimum* L. sprouts and microgreens? *Horticulture* **2022**, *8*, 98. [CrossRef]
78. Escobar-Bravo, R.; Klinkhamer, P.G.; Leiss, K.A. Interactive effects of UV-B light with abiotic factors on plant growth and chemistry, and their consequences for defense against arthropod herbivores. *Front. Plant Sci.* **2017**, *8*, 278. [CrossRef]
79. Thakur, K.; Kumari, C.; Zadokar, A.; Sharma, P.; Sarma, R. Physiological and omics-based insights for underpinning the molecular regulation of secondary metabolite production in medicinal plants: UV stress resilience. *Plant Physiol. Biochem.* **2023**, *204*, 108060. [CrossRef]
80. Dinan, L.; Dioh, W.; Veillet, S.; Lafont, R. 20-Hydroxyecdysone, from plant extracts to clinical use: Therapeutic potential for the treatment of neuromuscular, cardio-metabolic and respiratory diseases. *Biomedicines* **2021**, *9*, 492. [CrossRef] [PubMed]
81. Felipe, S.H.S.; Batista, D.S.; Chagas, K.; Correia, L.N.F.; Silva, T.D.; Fortini, E.A.; Silva, P.O.; Otoni, W.C. Accessions of Brazilian ginseng (*Pfaffia glomerata*) with contrasting anthocyanin content behave differently in growth, antioxidative defense, and 20-hydroxyecdysone levels under UV-B radiation. *Protoplasma* **2019**, *256*, 1557–1571. [CrossRef] [PubMed]
82. Schreiner, M.; Mewis, I.; Huyskens-Keil, S.; Jansen, M.A.K.; Zrenner, R.; Winkler, J.B.; O'Brien, N.; Krumbein, A. UV-B-induced secondary plant metabolites-potential benefits for plant and human health. *Crit. Rev. Plant Sci.* **2012**, *31*, 229–240. [CrossRef]
83. Jiménez-Aspee, F.; Theoduloz, C.; Pormetter, L.; Mettke, J.; Ávila, F.; Schmeda-Hirschmann, G. Andean *Prumnopitys andina* (Podocarpacae) fruit extracts: Characterization of secondary metabolites and potential cytoprotective effect. *Molecules* **2019**, *24*, 4028. [CrossRef] [PubMed]

84. Miliauskas, G.; van Beek, T.A.; de Waard, P.; Venskutonis, R.P.; Sudhölter, E.J. Identification of radical scavenging compounds in *Rhaponticum carthamoides* by means of LC-DAD-SPE-NMR. *J. Nat. Prod.* **2005**, *68*, 168–172. [CrossRef]
85. Biskup, E.; Lojkowska, E. Evaluation of biological activities of *Rhaponticum carthamoides* extracts. *J. Med. Plant Res.* **2009**, *3*, 1092–1098.