



Response of *Dendrobium officinale* Kimura et Migo, a Prized Medicinal Plant, to Continuous UV-B Irradiation at Different C/N Ratios

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Abstract

The response of *Dendrobium officinale* Kimura et Migo (*D. officinale*) to continuous UV-B irradiation at different carbon to nitrogen ratios (C/N ratios) was investigated. Seedlings grown for 60 days were incubated under aseptic conditions with UV-B irradiation ($15.6 \mu\text{W cm}^{-2}$) at different C/N ratios: control group (CK; C/N 30 without UV-B), UV-B + CK (C/N 30 with UV-B irradiation, similarly hereafter), UV-B + C/N 120, UV-B + C/N 60, UV-B + C/N 15, UV-B + C/N 10, UV-B + C/N 7.5. Growth parameters (the defoliation rate and the sprout number), photosynthetic pigments (carotenoids, chlorophyll a and chlorophyll b), total polysaccharides, total alkaloids, and activities of antioxidant enzymes were determined following 4, 8, 12, and 16 days of continuous UV-B exposure. Results indicated that UV-B irradiation increased the defoliation rate and the content of carotenoids, total polysaccharides and total alkaloids, as well as the activities of antioxidant enzymes. Conversely, UV-B irradiation reduced the sprout number and chlorophyll content in *D. officinale*. Compared with UV-B + CK, lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5) enhanced the defoliation rate and sprout number, but decreased antioxidant enzyme activities and total polysaccharide content during the whole period, and reduced total alkaloid content after 4 days of UV-B exposure. Following initial UV-B irradiation, lower C/N ratios increased the contents of carotenoid and chlorophyll b, while after 8 days, a reversal in carotenoid content was observed, and after 12 days, a reversal in chlorophyll b content was observed. Optimizing the C/N ratio (C/N 60) resulted in lower defoliation rate, higher photosynthetic pigments and total polysaccharides, and increased activities of antioxidant enzymes, whereas no significant change in sprout number and total alkaloid content was recorded under long-term UV-B irradiation. Furthermore, the UV-B + C/N 120 treatment negatively affected *D. officinale* in terms of an increased defoliation rate and reduced sprout number, photosynthetic pigments, and total alkaloids. Therefore, results suggested that an appropriate C/N ratio (C/N 60) could ameliorate the adverse effects of continuous UV-B irradiation on *D. officinale*.

Keywords *Dendrobium officinale* Kimura et Migo · UV-B irradiation · C/N ratio · Photosynthetic pigments · Antioxidant enzymes · Main active compounds

Introduction

Solar ultraviolet radiation (UV; 100–400 nm) is known as one of the most damaging agents that living organisms are exposed to (Grandahl et al. 2018). According to wavelength and biological effects, UV can be divided into UV-A (315–400 nm), UV-B (280–315 nm), and UV-C (100–280 nm). Although UV-B radiation is a minor portion of the solar UV radiation, it is possibly the most damaging component (Mazza et al. 2013). Currently, depletion of the stratospheric ozone layer, caused by the release of destructive chemicals, such as chlorofluorocarbons (CFCs), results in enhanced UV-B radiation reaching the Earth's surface (Fu

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and Shen 2017). Previous investigations have shown that increased UV-B could reduce growth and photosynthesis of plants; cause DNA damage and increase oxidative stress by inducing reactive oxygen species (ROS) synthesis, such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (Araujo et al. 2016; Cong and Li 2018; Singh et al. 2011a). There has been substantial research pertaining to the effects of elevated UV-B radiation on inhibition of growth and biomass of cotton (Dehariya et al. 2011), potato (Singh et al. 2011b), *Chrysanthemum* (Li et al. 2011), soybean (Ma et al. 2016), spinach and amaranthus (Singh et al. 2013). Recently the effects of UV-B irradiation on plant secondary metabolites have drawn great attention (Alonso et al. 2015; Kumari and Agrawal 2010; Kumari and Prasad 2013; Zhang and Bjorn 2009). For instance, Manaf et al. (2016) stated that a moderate extension of UV-B exposure time had a beneficial impact on the contents of caffeic acid and total phenols in coneflower (*Echinacea purpurea* (L.) Monech). Furthermore, the effects of UV-B irradiation on growth and the major active compounds might be attributed to protective mechanisms of plant, which can synthesize protective UV-B incorporating compounds, such as phenols and flavonoids, and initiate the activity of a free-radical scavenging system, such as increased antioxidant activities and induced low-molecular antioxidants (Dai et al. 2011; Martz et al. 2010; Singh et al. 2011a). It is also well documented that polysaccharides can act as free radical scavengers and inhibit DNA damage (Wong et al. 2011; Yang et al. 2017). The majority of previous studies have concentrated on the effects of short-term UV-B irradiation, while only a few have focused on continuous UV-B exposure (Schmidt et al. 2010). Carbon and nitrogen are the two essential nutrient elements for organisms, especially, nitrogen is the main constituent of amino acids, nucleotides, chlorophyll, alkaloids, and numerous other N-containing components (Zaid and Mohammad 2018). As for carbon to nitrogen (C/N) ratio, it has significant effects on the biomass productivity and nutritional components of microalgae (Lu et al. 2016) and carotenoids production of yeast (Braunwald et al. 2013), while effects on the plants have been less reported. Moreover, little is known about the responses of plants to continuous UV-B irradiation at different C/N ratios.

The dried stem of *D. officinale*, a prized traditional Chinese herb, has a long history of medicinal application, as recorded in a famous traditional Chinese medicine pharmacopeia, Shen Nong's Herbal Classic. It can improve immunity, and nourish the stomach and lungs (Ng et al. 2012). Previous reports indicate that polysaccharides and alkaloids, the major active compounds of *D. officinale*, have immunoregulation, hepato-protective and antioxidant functions; or anticancer; and neuroprotective effects (Ng et al. 2012; Xu et al. 2013). The biosynthesis and accumulation of polysaccharides and alkaloids in *D. officinale* is related to numerous

environmental factors, such as water, climate, mineral nutrition, and light (Lin et al. 2010; Zheng et al. 2012a, b). UV-B irradiation has enormous implications on field cultivation of *D. officinale*, but few studies have verified this. Furthermore, no studies have elucidated the effects of different C/N ratios on *D. officinale* under UV-B irradiation. Thus, the present research investigated the effects of continuous UV-B irradiation on *D. officinale* at different C/N ratios.

In the present study, we hypothesize that continuous UV-B irradiation may affect the growth of *D. officinale* in many ways, such as increasing of the defoliation rate; reducing the sprout number; altering the photosynthetic parameters; increasing the activity of antioxidant enzymes, and the amount of total polysaccharides and total alkaloids. Moreover, different C/N ratios may mitigate or exacerbate the effects of UV-B irradiation on *D. officinale*. Thus, it is beneficial to identify a moderate C/N ratio that may exert protective effects on *D. officinale* under UV-B exposure. In order to test the above hypothesis, we investigated the effects of artificial UV-B irradiation of $15.6 \mu\text{W cm}^{-2}$ on *D. officinale* at different C/N ratios under sterile conditions by determining their effects on growth; the activities of antioxidant enzymes; the content of photosynthetic pigments, total polysaccharides, and total alkaloids. Finally, we elucidated the optimal C/N ratio to alleviate the damage caused by continuous UV-B irradiation.

Materials and Methods

Surface sterilized seedlings of *D. officinale*, grown for 60 days, were provided by our laboratory (Key Laboratory of State Administration of Traditional Chinese Medicine for Production & Development of Cantonese Medicinal Materials). The optimal growth medium for seedlings was a basic solid 1/2 Murashige and Skoog medium (MS medium) with half-strength macronutrients and 30 g/L sucrose, supplemented with 2.0 mg/L naphthalene acetic acid (NAA). Sucrose served as the carbohydrate source and inorganic nitrogen (ammonium nitrate and potassium nitrate) served as the nitrogen source. The first treatment utilized 1/2 MS medium with a C/N ratio of 30, which contained 30 g/L sucrose, 0.289 g/L ammonium nitrate and 0.132 g/L potassium nitrate. The other treatments were designed by varying the nitrate nitrogen content of this basic medium while maintaining a consistent carbon content (Table 1). Seedlings characterized as green and healthy, with an average height of 5–6 cm, were chosen for incubation using the above treatment medium. All cultures were incubated at $25 \pm 2 \text{ }^\circ\text{C}$, under the intensity of 2000 lx from white fluorescent tubes (TL5- 28 W/1162 mm, Philips, China) with a 12: 12-h light: dark cycle. The distance between the seedlings and the white fluorescent tubes was 30 cm.

Table 1 Contents of sucrose, ammonium nitrate and potassium nitrate and the C/N ratio of the treatments

Treatments	Sucrose (g/L)	NH ₄ NO ₃ (g/L)	KNO ₃ (g/L)	C/N ratio
CK (C/N 30)	30	0.289	0.132	30:1
C/N 120	30	0.072	0.033	120:1
C/N 60	30	0.144	0.066	60:1
C/N 15	30	0.578	0.264	15:1
C/N 10	30	0.866	0.396	10:1
C/N 7.5	30	1.155	0.528	7.5:1

After 60 days for consistency, these cultures were divided into two groups: the control group (CK) and the experimental group. The control group was treated with C/N 30 without UV-B irradiation, while the experimental groups had differing C/N ratios with 15.6 $\mu\text{W cm}^{-2}$ UV-B irradiation: UV-B + C/N 120 (C/N 120; similarly hereafter), UV-B + C/N 60, UV-B + CK (C/N 30), UV-B + C/N 15, UV-B + C/N 10, UV-B + C/N 7.5. Supplemental UV-B was provided in doses of 15.6 $\mu\text{W cm}^{-2}$ (UV-B-313, LongPro co., LTD, China), for 4 h per day (from 12:00–16:00 h) for a sequential 16 days, imitating the exposure time of maximum UV-B intensity in shade in Guangdong province, during the *D. officinale* growth and development season. Each treatment had three replications. At 4, 8, 12, and 16 days after UV-B treatment, the followings were determined:

Growth Parameters

Growth parameters were determined in terms of brown leaves, the number of sprouts and the defoliation rate. A brown leaf was defined as a leaf in which greater than 50% of the leaf area turned brown. The number of sprouts was determined by the quantitative difference between the total numbers of buds before and after irradiation. The defoliation rate was calculated as follows: defoliation rate = (number of deciduous leaves + number of brown leaves) / total number of leaves.

Determination of Photosynthetic Pigments

Carotenoids (Car), chlorophyll a (Chl a), and chlorophyll b (Chl b) were extracted from 0.1 g blade with 5 mL 80% acetone. The extract was separated by centrifugation at 6000 $\times g$ for 10 min, and absorbances were recorded at 470, 646 and 663 nm by ultraviolet and visible spectrophotometer (UV755B, Shanghai APL instrument co., LTD, China). Contents of Car, Chl a, and Chl b were determined according to the formulas below (Wellburn and Lichtenthaler 1984):

$$\text{Chl a (mg/L)} = (12.21 * A_{663}) - (2.81 * A_{646})$$

$$\text{Chl b (mg/L)} = (20.13 * A_{646}) - (5.03 * A_{663})$$

$$\text{Car (mg/L)} = (A_{470} * 1000 - 3.27 * \text{Chl a} + 104 * \text{Chl b}) / 229$$

A = absorbance.

Determination of Antioxidant Enzyme Activities

Fresh leaf blades (0.5 g) were ground with 5 mL 0.1 M phosphate buffer (pH 6.8) on an ice-bath. The homogenate was centrifuged at 12,000 $\times g$ for 15 min at 4 °C. The supernatant was used as the enzyme extract for assays for superoxide dismutase, guaiacol peroxidase (POD) and catalase (CAT) activity. SOD activity was determined following the method of Beyer and Fridovich (1987). Briefly, the reaction solution contained 50 mM sodium phosphate buffer, 2.1 mM methionine, 100 μM ethylene diamine tetraacetic acid (EDTA), 1.72 mM nitroblue tetrazolium chloride (NBT), 0.24 mM riboflavin, and 50 μL enzyme extract. The solution was allowed to react for 20 min under fluorescent light in 4000 lx and then kept in the dark immediately. The reference solution in the absence of enzyme was placed in the dark as a control group (CK). Finally, the absorbance was determined at 560 nm, SOD activity was calculated as follows:

$$\text{SOD activity [U/g (FW)]} = (A_{\text{CK}} - A_{\text{R}}) * V / (0.5 * A_{\text{CK}} * W * V_{\text{R}})$$

A_{CK} = absorbance of the reference solution, A_{R} = absorbance of the reaction solution, V = volume of the extracting solution, V_{R} = volume of the reaction solution used for determination at 560 nm, W = weight of the fresh leaf blades.

One unit of SOD was defined as the quantity of enzyme required to inhibit the rate of NBT photoreduction by 50%. POD and CAT activities were estimated by the method of Chance and Maehly (1955), with some modification: the POD reaction liquid consisted of 50 mM sodium phosphate buffer (pH 7.8, Tianjin damao chemical reagent factory, China), 200 mM hydrogen peroxide (H₂O₂, Tianjin zhiyuan chemical reagent factory, China), 50 mM guaiacol (CAS: 90-05-1, Aladdin, China), and enzyme extract. The absorbance of the reaction liquid was recorded at 470 nm within 3 min. The POD activity was calculated as follows:

$$\text{POD activity [U/g (FW)]} = \Delta A_{470} * V / (0.01 * V_{\text{R}} * W * t)$$

ΔA_{470} = changes in absorbance during the reaction time, V = volume of the extracting liquid, V_{R} = volume of the reaction liquid used for determination at 470 nm, W = weight of fresh leaf blades, t = reaction time.

One unit of POD was defined as the change in the absorbance of the reaction liquid by 0.01 at 470 nm. The CAT reaction solution contained 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 200 mM H₂O₂ and enzyme extract. The absorbance was recorded at 240 nm. CAT activity was determined as follows:

$$\text{CAT activity [U/g (FW)]} = \Delta A_{240} * V / (0.01 * V_{\text{R}} * W * t)$$

ΔA_{240} = the change in absorbance during the reaction time, V = volume of the extracting solution, V_R = volume of the reaction solution used for determination at 240 nm, W = weight of fresh leaf blades, t = reaction time.

One unit of CAT was defined as the change in the absorbance of the reaction solution by 0.01 at 240 nm.

Determination of Total Polysaccharides and Total Alkaloids

Total polysaccharides were determined according to the China Pharmacopeia Committee (2015). Dry stem (2.0 g) was crushed in 2 mL distilled water with a mortar and pestle. The homogenate, with 200 mL distilled water, was refluxed for 2 h and allowed to cool to ambient temperature. The extract was filtrated and transferred to a 250-mL volumetric flask. A 6-mL aliquot from the 250-mL crude extract was centrifuged ($4000\times g$) with 30 mL ethanol for 30 min. The precipitate was collected and washed twice with 80% ethanol (24 mL) and centrifuged again. The resulting precipitate was redissolved in boiling water and transferred to a 10-mL volumetric flask as purified extract. A 1-mL aliquot of the extracts was used for total polysaccharides determination at 488 nm.

Total alkaloids were measured according to Zheng et al. (2012a). Briefly, 0.4 g dry stem was soaked in ammonia solution (30 min) and refluxed with 10 mL chloroform for 2 h. The extract was filtered, and then 5 mL of the filtrate was transferred to a separatory funnel with 5 mL chloroform, 5 mL buffer (pH 4.5) and 1 mL 0.04% bromocresol green solution. Afterward, the upper fraction from the separatory funnel was filtered. A 5-mL aliquot of the filtrate was contained with 1 mL 0.01 mol/L NaOH for determination. The absorbance was recorded at 620 nm.

Statistical Analysis

All experiments were replicated three times. The data were subjected to one-way analysis of variance (ANOVA) using SPSS statistical software package (ver. 23.0). Differences of treatment means were compared by the Duncan's multiple range tests at $P < 0.05$.

Results

Growth Parameters

Compared with CK, UV-B irradiation caused damage to *D. officinale* blades in terms of the increased defoliation rate under UV-B + CK, with an increasing defoliation rate observed upon prolonged UV-B exposure (Figs. 1a, 2). Treatment with C/N 60 to UV-B treated *D. officinale*

seedlings (UV-B + C/N 60) decreased the defoliation rate in comparison with UV-B + CK, while other treatments increased the defoliation rate even further. Especially under lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5), the defoliation rate was higher as treatment C/N ratios decreased over the entire UV-B irradiation period. After 16 days of irradiation, the defoliation rate was 60.67, 32.73, 44.64, 61.65, 60.10 and 84.07% for C/N ratios from 120 to 7.5 (UV-B + C/N 120, UV-B + C/N 60, UV-B + CK, UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5), respectively. This result showed that UV-B + C/N 60 had a lower defoliation rate and more positive effect on UV-B treated plants during the whole period.

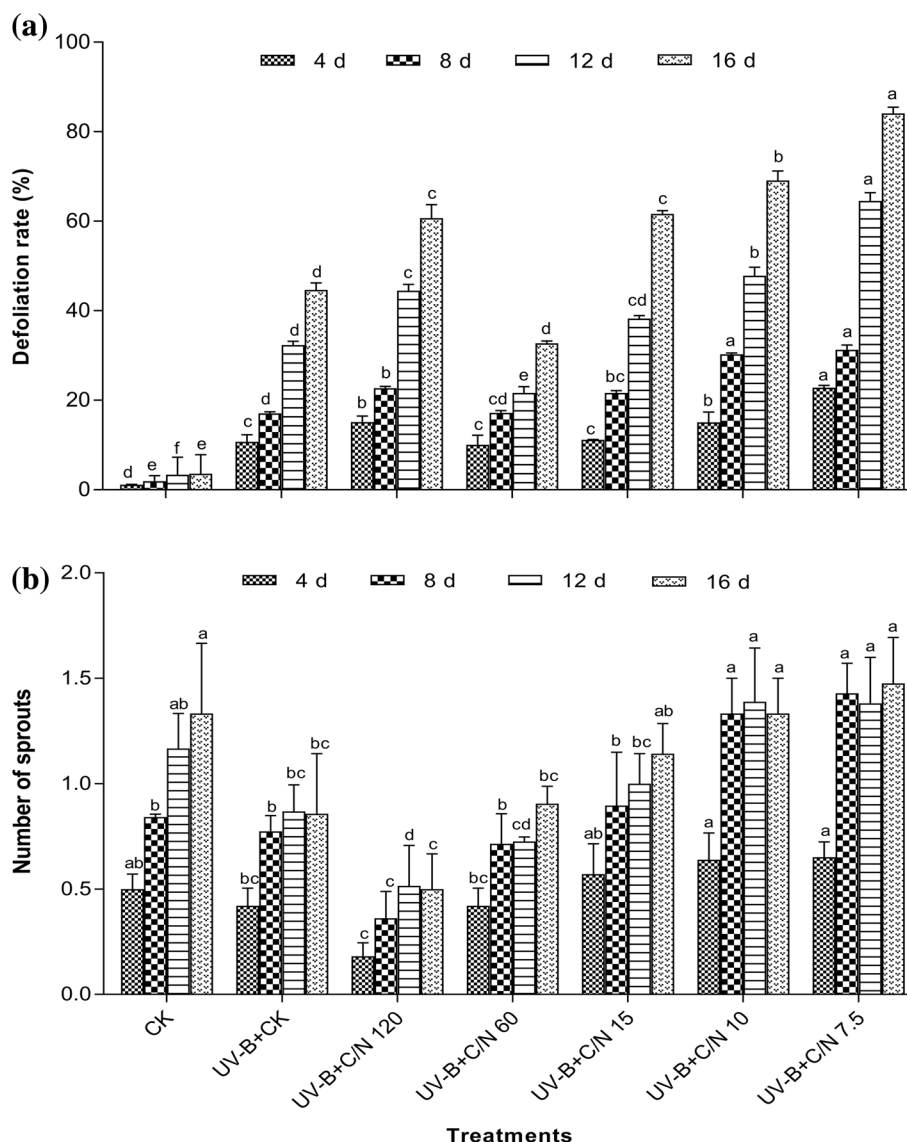
The number of sprouts decreased after exposure to UV-B irradiation, with the decrease in sprouts becoming more pronounced as exposure time increased. Treatment UV-B + C/N 60 showed no significant differences with the UV-B + CK during the whole period. However, lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5) led to an increase in the number of sprouts, when compared to UV-B + CK, regardless of the UV-B exposure duration (4, 8, 12 or 16 days) (Figs. 1b, 2). After 16 days of UV-B exposure, the average number of sprouts was 0.500, 0.905, 0.857, 1.143, 1.333, and 1.147, for C/N ratios from 120 to 7.5 (UV-B + C/N 120, UV-B + C/N 60, UV-B + CK, UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5), respectively.

Photosynthetic Pigments

Compared with CK, treatment UV-B + CK induced a reduction in the Chl a content by 18.38, 7.66, 13.57, and 21.25% after 4, 8, 12, and 16 days of UV-B irradiation, respectively (Fig. 3a). During 8 days of UV-B exposure, with the exception of UV-B + C/N 120, there was no significant increase under the other treatments as compared to UV-B + CK. However, after 16 days of UV-B exposure, plants under treatment UV-B + C/N 60 improved Chl a content by 15.52% with respect to that of the plants under UV-B + CK (Fig. 3a).

The Chl b content was also negatively influenced by UV-B irradiation and it significantly decreased by 29.11, 15.81, 31.70, and 28.66% after 4, 8, 12, and 16 days of UV-B exposure, respectively, in treatment UV-B + CK as compared to that of CK (Fig. 3b). However, this effect could be ameliorated by treatment UV-B + C/N 60 after 4 days (4.75%), 8 days (4.02%), 12 days (18.35%), and 16 days (26.62%) of UV-B exposure relative to UV-B + CK. Furthermore, lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10, and UV-B + C/N 7.5) increased the Chl b content during 12 days of UV-B exposure and then decreased at 16 days, but treatment UV-B + C/N 120 decreased the Chl b content during the whole period, compared with UV-B + CK (Fig. 3b).

Fig. 1 Effects of UV-B irradiation on the defoliation rate (a) and the number of sprouts (b) of *D. officinale* seedlings at different C/N ratios. Data expressed as mean \pm SD ($n=3$). Data followed by different letters indicate significant differences between means within each parameter (4, 8, 12, and 16 days) according to the Duncan's multiple range tests ($P < 0.05$)



UV-B irradiation in UV-B + CK enhanced the Car content by 21.40, 25.48, 17.09, and 24.21% after 4, 8, 12, and 16 days of UV-B exposure, respectively, compared with CK (Fig. 3c). Lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5) increased the Car content during 8 days of UV-B exposure and then decreased at the following days as compared to UV-B + CK. During the initial stage of UV-B irradiation, the Car content showed a non-significant change under treatment UV-B + C/N 60, while after 8 days, it increased by 16.31% (12 days) and 9.52% (16 days), compared with UV-B + CK (Fig. 3c). These results showed that a suitable C/N ratio (i.e., C/N 60) was probably beneficial to chlorophyll synthesis under prolonged UV-B irradiation and may aid in resisting UV-B damage to a maximum degree (Fig. 3a–c).

Activities of Antioxidant Enzymes

UV-B irradiation enhanced the activity of SOD by 147.77, 32.65, 2.93, and 4.70% after 4, 8, 12, and 16 days of UV-B irradiation in treatment UV-B + CK when compared with the control (Fig. 4a). Lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10, and UV-B + C/N 7.5) caused decrease in SOD activity during the whole period, with respect to that of the UV-B + CK. However, treatments UV-B + C/N 60 and UV-B + C/N 120 increased the SOD activities by 96.34 and 71.98%, respectively, after 12 days of UV-B exposure, and by 63.67% and 21.32%, respectively, after 16 d of UV-B exposure, as compared to UV-B + CK (Fig. 4a).

POD activity also significantly increased by 171.30, 93.48, 58.52, and 96.67% in response to 4, 8, 12, and 16 days of UV-B exposure in treatment UV-B + CK compared

Fig. 2 Effects of UV-B irradiation on the growth of *D. officinale* seedlings at different C/N ratios

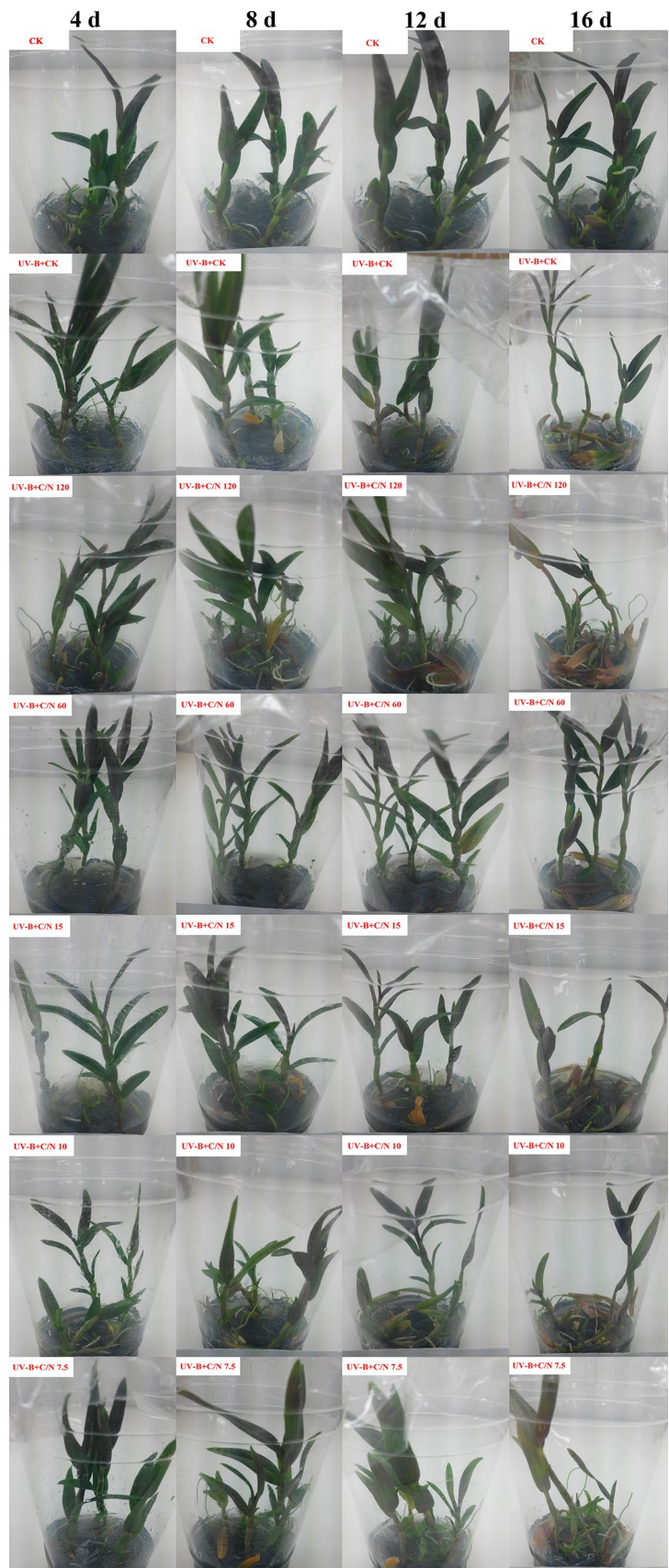
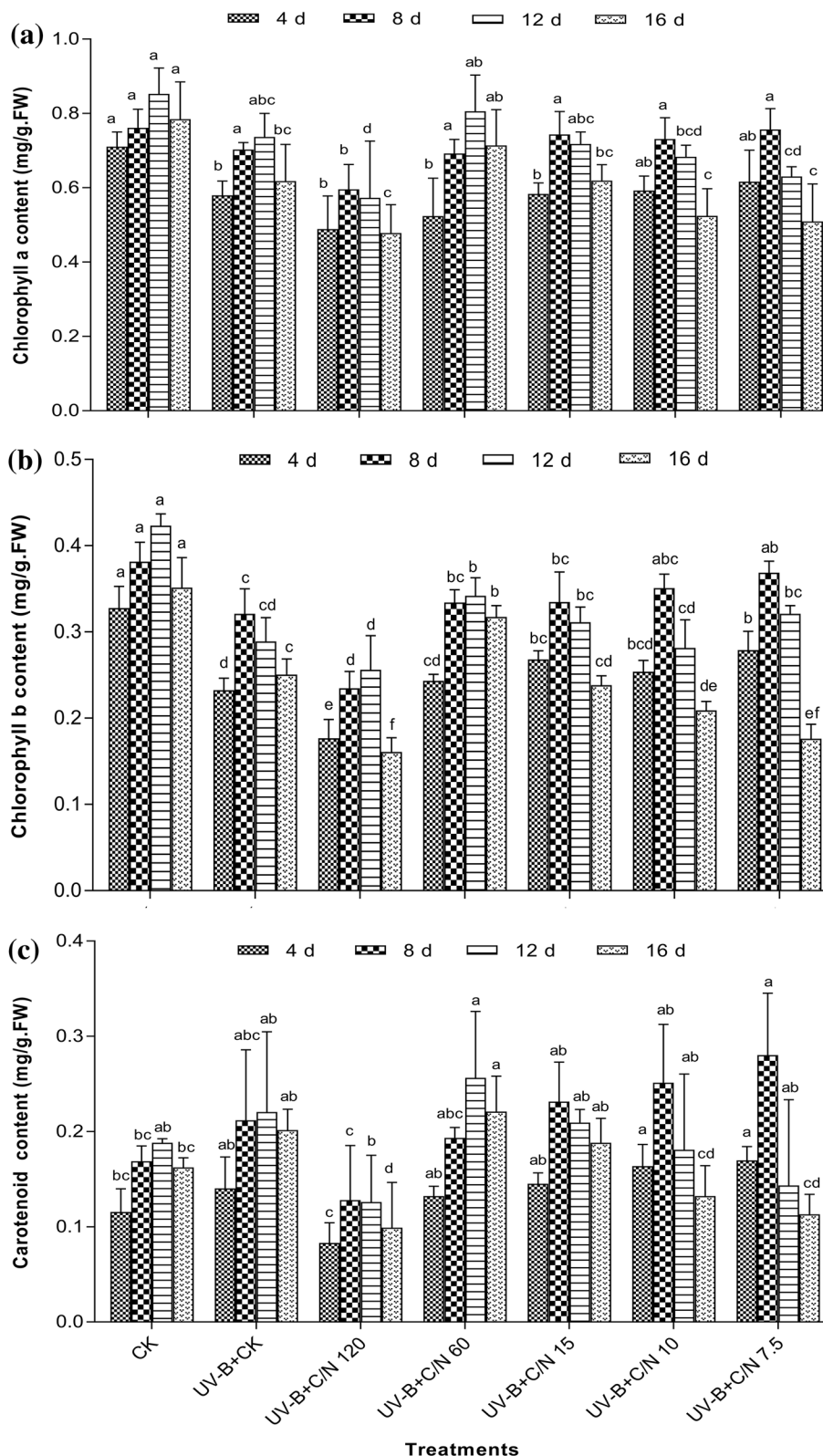


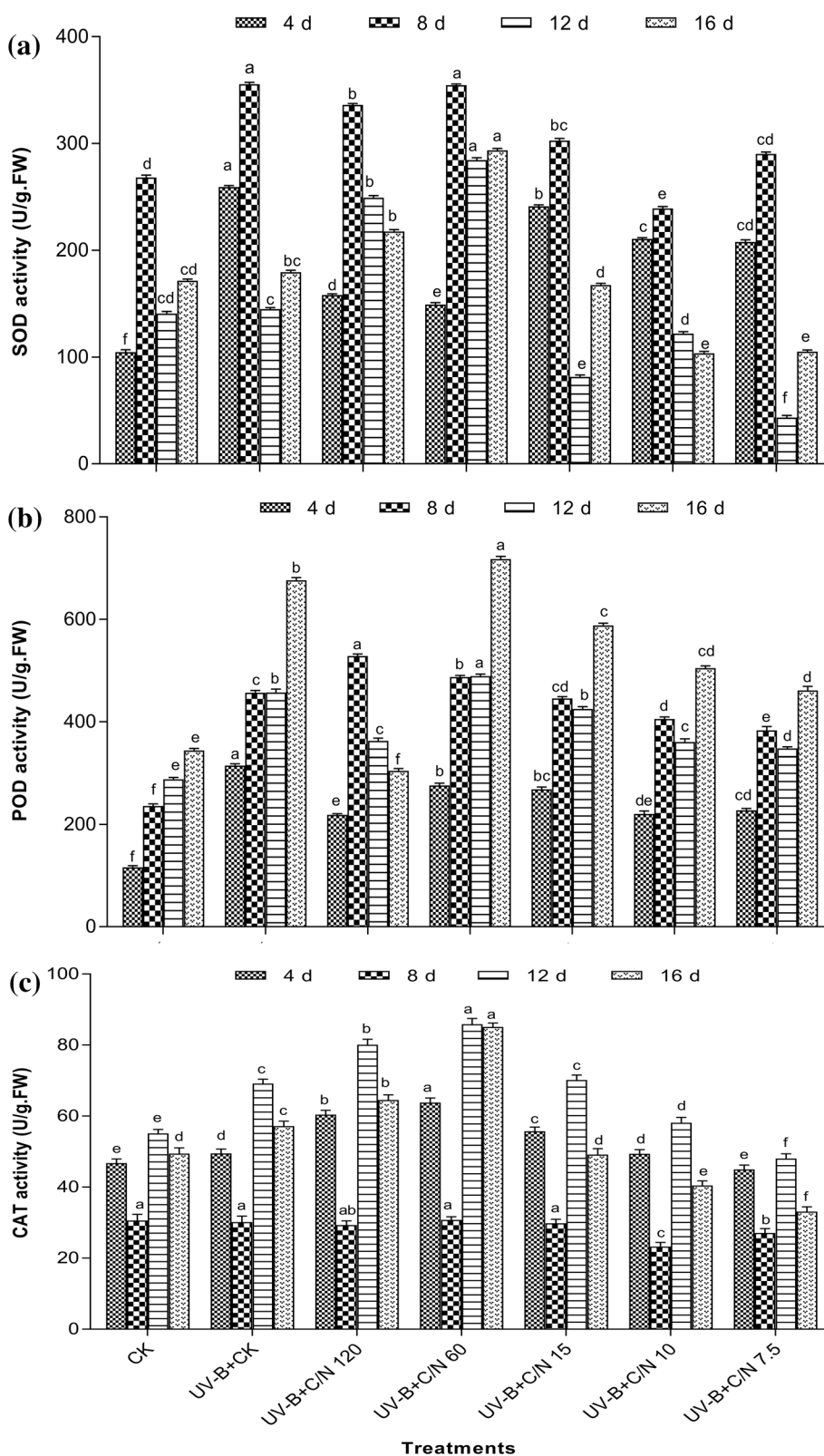
Fig. 3 Effects of UV-B irradiation on the content of chlorophyll a (a), chlorophyll b (b) and carotenoid (c) in *D. officinale* seedlings at different C/N ratios. Data expressed as mean \pm SD ($n=3$). Data followed by different letters indicate significant differences between means within each parameter (4, 8, 12, and 16 days) according to the Duncan's multiple range tests ($P < 0.05$)



to that of the corresponding control (Fig. 4b). Treatment UV-B + C/N 60 further increased the POD activity by 6.69, 7.07, and 6.15% after 8, 12, and 16 days of UV-B irradiation,

respectively, whereas the POD activity was reduced under the other C/N ratio treatments, compared with UV-B + CK (Fig. 4b).

Fig. 4 Effects of UV-B irradiation on the activity of SOD (a), POD (b) and CAT (c) of *D. officinale* seedlings at different C/N ratios. Data expressed as mean \pm SD ($n=3$). Data followed by different letters indicate significant differences between means within each parameter (4, 8, 12, and 16 days) according to the Duncan’s multiple range tests ($P < 0.05$)



With the exception of a non-significant change at 8 days UV-B exposure, CAT activity was enhanced by 5.84, 25.45, and 16.67%, respectively, in response to 4, 12, and 16 days of

UV-B irradiation in treatment UV-B + CK as compared with that of control plants (Fig. 4c). Moreover, the CAT activities decreased under treatments UV-B + C/N 10 and UV-B + C/N

7.5 but increased under other treatments (UV-B + C/N 120, UV-B + C/N 60, and UV-B + C/N 15) in comparison with treatment UV-B + CK. Especially, treatment UV-B + C/N 60 resulted in the maximum increase in CAT activity by 29.01, 2.10, 24.21, and 48.79%, respectively, after 4, 8, 12, and 16 days of UV-B exposure relative to treatment UV-B + CK (Fig. 4c).

Total Polysaccharides and Total Alkaloids

UV-B irradiation enhanced the accumulation of polysaccharides by 35.45, 18.29, 115.65, and 19.55%, respectively, after 4, 8, 12, and 16 days of UV-B exposure in treatment UV-B + CK with respect to CK (Table 2). Moreover, total polysaccharides were further enhanced in higher C/N ratio treatments (UV-B + C/N 120 and UV-B + C/N 60) but not in lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10, and UV-B + C/N 7.5), as compared to UV-B + CK. A maximum content of total polysaccharides was observed in treatment UV-B + C/N 60, after 16 days of UV-B irradiation (Table 2).

The total alkaloid content of *D. officinale* significantly increased by 77.68, 212.38, 101.30, and 52.84%, respectively, after 4, 8, 12, and 16 days of UV-B exposure in treatment UV-B + CK compared with CK (Table 3).

Following initial UV-B exposure (4 days of UV-B exposure), an increase of total alkaloid content by 117.80, 48.51, and 64.39% was found under lower C/N ratio treatments (UV-B + C/N 15, UV-B + C/N 10, and UV-B + C/N 7.5), respectively, after which the total alkaloid content decreased relative to UV-B + CK. There was a significant ($P < 0.05$) reduction of total alkaloid content between UV-B + C/N 120 and UV-B + CK, whereas no significant difference ($P > 0.05$) was noticed between UV-B + C/N 60 and UV-B + CK (Table 3).

Discussion

UV-B irradiation has been shown, in the majority of studies, to significantly affect growth and synthesis of secondary metabolites (Interdonato et al. 2011; Kumari et al. 2009; Matsuura et al. 2013; Takshak and Agrawal 2014). Our experiments show that UV-B exposure inhibited growth of *D. officinale* as depicted by the browning of leaves, increased defoliation and reduced number of sprouts. These inhibitory effects were more prominent with prolonged UV-B irradiation (Fig. 1). It is possible that foliage, as the most sensitive organ of plant, turns to brown and deciduous to reduce UV-B penetration into leaf. This result is consistent with

Table 2 Effects of UV-B irradiation on the content of total polysaccharides in dry stem of *D. officinale* at different C/N ratios

Treatment	The content of total polysaccharides ($\mu\text{g/g}$)			
	4 days	8 days	12 days	16 days
CK	3833.96 \pm 315.23cd	4904.25 \pm 154.81c	4443.8 \pm 184.49c	5151.22 \pm 355.89cd
UV-B + CK	5193.04 \pm 127.34abc	5801.03 \pm 140.18c	9582.90 \pm 121.97ab	6158.26 \pm 118.25bc
UV-B + C/N 120	6122.34 \pm 244.87a	10424.32 \pm 245.71a	10423.01 \pm 195.45a	7990.12 \pm 297.58b
UV-B + C/N 60	5567.56 \pm 57.34ab	7464.06 \pm 227.76b	11630.81 \pm 461.42a	11378.94 \pm 281.15a
UV-B + C/N 15	4514.14 \pm 120.08bc	3072.14 \pm 199.40d	7542.49 \pm 140.90b	3758.88 \pm 270.77de
UV-B + C/N 10	2847.60 \pm 143.25d	1983.49 \pm 66.26d	3232.96 \pm 122.59cd	1757.46 \pm 266.12f
UV-B + C/N 7.5	1012.49 \pm 190.42e	1677.16 \pm 132.35d	1767.77 \pm 140.16d	903.90 \pm 138.35f

Data expressed as mean \pm SD ($n=3$). Different letters within columns indicate significant differences according to the Duncan's multiple range tests ($P < 0.05$)

Table 3 Effects of UV-B irradiation on the content of total alkaloids in dry stem of *D. officinale* at different C/N ratios

Treatment	The content of total alkaloids ($\mu\text{g/g}$)			
	4 days	8 days	12 days	16 days
CK	18.46 \pm 4.23d	8.56 \pm 1.27c	15.37 \pm 1.44de	19.57 \pm 1.41b
UV-B + CK	32.80 \pm 2.37c	26.74 \pm 0.44b	30.94 \pm 1.70a	29.91 \pm 1.48a
UV-B + C/N 120	8.72 \pm 0.62e	10.78 \pm 1.07c	11.79 \pm 6.16f	5.62 \pm 3.62c
UV-B + C/N 60	29.43 \pm 1.60c	21.18 \pm 1.35b	21.00 \pm 3.20bcd	30.37 \pm 1.09a
UV-B + C/N 15	69.47 \pm 6.96a	19.23 \pm 1.1b	23.87 \pm 0.79abc	34.31 \pm 2.40a
UV-B + C/N 10	48.71 \pm 4.95b	20.88 \pm 2.94b	17.67 \pm 2.70cde	6.47 \pm 5.47c
UV-B + C/N 7.5	53.92 \pm 1.83b	32.68 \pm 2.17a	25.48 \pm 1.33ab	4.13 \pm 1.40c

Data expressed as mean \pm SD ($n=3$). Different letters within columns indicate significant differences according to the Duncan's multiple range tests ($P < 0.05$)

earlier studies on cotton plants where plant height and leaf area decreased under ambient UV-B irradiation (Dehariya et al. 2011). However, little is known about the combined effects of C/N ratios and UV-B irradiation on the growth and quality of *D. officinale*. Our research demonstrates that different C/N ratios might alter the deleterious effects of UV-B irradiation. A decrease in the defoliation rate and a non-significant change of the number of sprouts was observed in treatment C/N 60 under continuous UV-B exposure (Fig. 1). Thus, these results suggest that a moderate C/N ratio could counteract the adverse effects of UV-B exposure on *D. officinale* growth. More detailed discussions are required on the physiological and biochemical aspects to determine the exact mechanism of mitigation associated with the moderate C/N ratio.

UV-B irradiation is known to cause significant effects, not only on growth, but also on other physiological characteristics, such as photosynthetic pigments and antioxidant enzymes (Baroniya et al. 2013; Gonzalez et al. 2009; Xu et al. 2008). In the present study, UV-B exposure reduced the content of chlorophyll but enhanced the content of carotenoids, which is consistent with previous studies on *Cymbopogon citratus* (D.C.) Staph, *Prunus dulcis*, amaranthus, and spinach (Kumari and Agrawal 2010; Ranjbarfordoei et al. 2011; Singh et al. 2013). Several explanations could be given for the reduction of chlorophyll content, including degradation of chlorophyll due to the destruction of related enzymes; inhibition of photosynthetic pathway; damage to the photosynthetic capacity of chloroplasts; and decrease in the seedlings' height and leaf area (Dehariya et al. 2011; Ranjbarfordoei et al. 2011). In addition, carotenoids protect the photosynthetic system from photo-damage by absorbing excess excitation energy, thus increased carotenoid content could alleviate the damage caused by UV-B exposure on chlorophyll (Agrawal and Rathore 2007). The response of *D. officinale* to continuous UV-B irradiation on the photosynthetic pigments was variable for seedlings incubated at different C/N ratios. Nitrogen, as an essential factor for the photosynthetic pigments, can stimulate pigment synthesis by facilitating N metabolism (Xu et al. 2014). In the present investigation, the photosynthetic pigment contents were augmented in treatments with lower C/N ratios (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5) during 8 days of UV-B exposure, while following prolonged exposure, a reduction in pigments was observed. Conversely, chlorophyll and carotenoid contents increased in the UV-B + C/N 60 treatment under long-term UV-B exposure, which suggests that suitable C/N 60 ratio contributed to mitigation of UV-B-induced damage on *D. officinale* (Fig. 3).

Antioxidant enzymes, which scavenge free radicals and help alleviate oxidative stress, were affected by UV-B irradiation. Synthesis of antioxidant enzymes might be provoked by UV-B to maintain the metabolic stability of plants in the

presence of increased ROS production (Wang et al. 2015). This was validated by our results, in which higher activities of antioxidant enzymes (SOD, POD, and CAT) were observed after exposure of *D. officinale* to UV-B radiation (Fig. 4). Similar findings were also reported by Takshak and Agrawal (2014) in *Withania somnifera*, Wang et al. (2015) in *Oryza sativa* L. and Yannarelli et al. (2006) in *Helianthus annuus* L. Furthermore, different C/N ratio treatments also had differing effects on the activities of these antioxidant enzymes. Earlier investigations reported that the activities of SOD and CAT were markedly higher under low nitrogen conditions in coffee leaves (Pompelli et al. 2010). Our study also showed that the activities of antioxidant enzymes were higher under continuous UV-B irradiation with higher C/N ratios, while the C/N 60 treatment ameliorated the oxidative damage caused by long-term UV-B irradiation to the highest degree (Fig. 4).

Polysaccharides and alkaloids are the main bioactive compounds of *D. officinale* (Zheng et al. 2012a). Previous studies indicate that the quality of medicinal plants could be enhanced under UV-B exposure (Sun et al. 2010). In our study, UV-B irradiation improved the total polysaccharide and alkaloid contents. Compared with CK, a highest increase in total polysaccharide content was found at 12 days exposure to UV-B (Table 2). The increase in polysaccharide accumulation might be related to its anti-UV-B effects (Wong et al. 2011). UV-B irradiation also led to an augmentation of the alkaloid synthesis, which might be due to its effective quenching of ROS induced by UV-B (Paranhos et al. 2009). This result agrees with (Pi et al. 2010), where an 11-fold enhancement in camptothecin content was found under UV-B irradiation of *Camptotheca acuminata*. Additionally, (Zu et al. (2010)) reported that long-time UV-B exposure significantly increased the content of taxol in *Taxus chinensis* var. *mairei*. Moreover, the total polysaccharide and alkaloid contents in *D. officinale* were also altered under different C/N ratios. Total polysaccharides increased with higher C/N ratios under long-term UV-B irradiation, with a maximum content observed at C/N 60 after 16 days. Conversely, total alkaloids showed a non-significant change with C/N 60 ratio under continuous UV-B exposure, with maximum content being observed at C/N 15 after 16 days of UV-B exposure. Polysaccharides are complex carbohydrates and are derived as a part of plant primary metabolism while alkaloids are biosynthesized as a part of plant secondary metabolism. The inverse trend of total polysaccharides and alkaloids might suggest a trade-off between plant primary and secondary metabolism.

In conclusion, UV-B exposure led to a reduction in growth (the increasing defoliation rate and the decreasing sprout number) and photosynthesis (chlorophyll content) compared with CK, while increased the content of total polysaccharides and total alkaloids, as well as the activities

of antioxidant enzymes (SOD, POD, and CAT). The treatment UV-B + C/N 120 might aggravate the negative effects on *D. officinale*, such as increasing the defoliation rate and decreasing the sprout number, the activities of antioxidant enzymes, the content of photosynthetic pigments, and total alkaloids compared with UV-B + CK during the whole period. Lower C/N ratios (UV-B + C/N 15, UV-B + C/N 10 and UV-B + C/N 7.5) increased the defoliation rate and sprout number but decreased the content of total polysaccharides and activities of antioxidant enzymes compared with UV-B + CK over the entire UV-B irradiation period. At the beginning, lower C/N ratios increased the content of photosynthetic pigments and total alkaloids, while following prolonged exposure, a reduction was found in total alkaloid content after 4 days, in carotenoid content after 8 days, and in chlorophyll content after 12 days. However, a moderate C/N ratio (C/N 60) could alleviate the negative effects on *D. officinale*. The protective strategies associated with C/N 60 under UV-B stress mainly include reduction of defoliation; increment of chlorophyll and carotenoid contents; enhanced activities of antioxidant enzymes (SOD, POD, and CAT); accumulation of total polysaccharides; and the maintenance of total alkaloids. Further studies should be conducted to find the optimal cultivation conditions to enhance the yield and quality of *D. officinale*.

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Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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