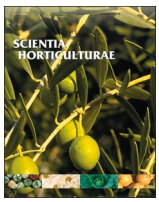
Scientia Horticulturae 322 (2023) 112415

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti



Investigating physiological responses of Wild Rocket subjected to artificial Ultraviolet B irradiation

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ARTICLE INFO

Keywords:
UV-B
Diplotaxis tenuifolia
phytochemicals biosynthesis
photobiological responses
lipid peroxidation
abiotic stress

ABSTRACT

Utilization of ultraviolet-B radiation (UV-B) is a way to encourage the accumulation of phytochemicals such as glucosinolates, carotenoids, phenolics and flavonoids in plants. In the current research, UV-B treatments were performed, as a possible elicitation strategy to promote the secondary metabolites accumulation in wild rocket (*Diplotaxis tenuifolia* (L.) DC*)*. After performing the destructive analysis, it has been found that the concentration of chlorophyl *a* and *b* as well as carotenoids decreased in response to the UV-B stress. Thiobarbituric acid reactive substances (TBARS) assay has been performed to investigate the membranes damage caused by the UV-B radiation. The analyses confirmed that the highest UV-B tested dose induced the greater damage. Interestingly, an increase of phenolic index, and an accumulation of anthocyanins and glucosinolates has been recorded under the successive exposure of varying UV-B doses. The total sugars, reducing sugars and sucrose buildup were also remarkable. Non-destructive analysis revealed a decline in the overall performance of the UV-B treated plants as well as the maximum quantum efficiency of photosystem II. A decrease in the chlorophyll estimates while an enhanced flavanols and anthocyanins were observed through non-destructive readings. Results of this study not only provided a feasible UV-B dose selection and supplementation for the wild rocket but also the understanding about enhanced phytochemical accumulation in plants against UV-B stress.

1. Introduction

The subject of ultraviolet radiation reaching earth has received immense attention in recent decades due to stratospheric ozone layer depletion. The sun radiates ultraviolet radiation, which is separated into three bands called UV-A (315–400 nm), UV-B (280–315 nm), and UV-C. (200–280 nm). Even though UV-B radiation only makes up a small part of solar radiation that reaches the planet, it is essential for numerous biological activities taking place worldwide. In order to develop crops in a way that produces enough food, fiber, and other raw materials in response to rising UV-B levels, it is necessary to have a thorough understanding of the UV-B impacts on crop species. In general, UV-B leads to oxidative stress and a variety of impacts, including damage to plant tissue, molecules, and even individual cells. High UV-B exposures disrupt proteins, lipids, membranes, and DNA, which causes an adverse effect on plant growth and development (Artes et al. 2009). Plants produce secondary metabolites, such as antioxidants, as a form of defense that enhances ROS detoxification and stress resistance (Khaleghi et al. 2019). In other words, environmental stimuli are thought to be a viable method of enhancing antioxidants and phytochemicals because numerous secondary metabolites production is typically related to plant responses to environmental challenges.

To assess the stress response of the plants in response to UV-B radiation, two signaling pathways have been suggested by the scientists. One such mechanism, which is not UVB-specific, suggests that UVB-induced oxidative stress responses may be caused by molecular breakdown and/or an accumulation of signaling molecules like ROS and molecules involved in wound or defense, like jasmonic acid, salicylic acid, nitric oxide, and ethylene. Consequently, stress-associated genes, which are often activated by signaling pathways related to injury and defense, become overexpressed (e.g., PR-1, PR-2, PR-5 and the defense gene PDF1.2) (Cisneros-Zevallos et al. 2014). Contrarily, another signaling

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https://doi.org/10.1016/j.scienta.2023.112415
Received 10 May 2023; Received in revised form 22 July 2023; Accepted 10 August 2023
Available online 16 August 2023

pathway which is UVB-specific and leads to UV-B protection and morphological alterations in plants is managed by cytosolic UVR8 photoreceptor. In this type of signaling, UVR8 links with the multi-functional E3 ubiquitin ligase constitutively photomorphogenic 1 (COP1), monomerizes, and translocate into the nucleus where it suppresses the degradation of the photomorphogenic transcription factor elongated hypocotyl 5 (HY5). A variety of essential components involved in UV acclimation response and UV protection are successively regulated by HY5 and its homolog (*HYH*), including genes encoding the phenylpropanoid pathway enzymes phenylalanine ammonia lyase (*PAL*), chalcone synthase (*CHS*), and flavonol synthase (*FLS*) (Brown and Jenkins, 2008). Increased UV-B radiation in numerous studies has been proven to have a major impact on the morphological (barley) (Klem et al. 2012), physiological (pigeon pea) (Gai et al. 2022), and molecular processes (*Arabidopsis*) (Heijde and Ulm, 2012) of plant species as highlighted in Fig. 1.

Wild Rocket (*Diplotaxis tenuifolia* (L.) DC.) is a widely spread baby leaf vegetable consumed for the source of fibers, vitamins and antioxidants. Numerous research projects have been carried out to assess the nutrient profiles of vegetables and fruits especially associated with the decrease risks of cardiovascular diseases and cancer (Hord et al. 2009). This leafy vegetable, which is a member of the Brassicaceae family, is prized for its unusual sensory qualities while also being an excellent source of phytonutrients, such as glucosinolates. In the current study, secondary metabolites of wild rocket were investigated after growing it indoor in a glasshouse under LED lights. For UV-B exposure plants were moved from glasshouse to a UV-B chamber where a fixed wavelength of 315 nm was administered on to the wild rocket plants assisted with varying cumulative UV-B doses of 21.6 KJ/m^2, 43.2 KJ/m^2, 86.4 KJ/m^2 and 172. 8 KJ/m^2. All the UV-B treatments were performed at night time by considering that in darkened leaves, UV-B treatment delays the appearance of senescence symptoms, when followed by light treatment (Sztatelman et al. 2015). Moreover, UV-B is dangerous if comes in contact with human, so in order to eradicate the chances of human contact during the day time and to better appreciate responses that were uniquely due to the UV-B exposure and its effects. The aim of the study was to record the response of wild rocket towards different UV-B doses and to check whether or not UV-B will assist wild rocket in increase secondary metabolites accumulation. Non-destructive estimation of maximum quantum yield of photosystem II (Fv/Fm) and performance index (PI) were recorded by measuring the chlorophyll *a* fluorescence. Moreover, analysis of chlorophyl *a* and *b*, carotenoids, nitrates, total sugars, reducing sugars, sucrose, glucosinolates, phenolic index, anthocyanins as well as Thiobarbituric acid reactive substances (TBARS) were performed.

2. Materials and methods

2.1. Plant material

Wild rocket (*Diplotaxis tenuifolia* 'Atlanta') was used as planting material and its seeds were sown in pots filled with a peat-based substrate. The expected plant density was kept around 1150 plants/m^2 and the number of seeds utilized was equivalent to about 3 kg/ha. Cultivation took place in an experimental greenhouse during spring summer cycle for both experiments under monitored growing conditions (24 ± 2°C) with the addition of supplemental LED lights (photoperiod of 16h) with an average PPFD of 65 μmole s^{-1} m^{-2} (maximum: 110 μmole s^{-1} m^{-2} and minimum 30 μmole s^{-1} m^{-2}). The composition of LED recipe used was (R: 77.1%; G+Y: 17.9%; B: 5%) as shown in Fig. 2, while the plants were watered and fertilized regularly.

2.2. Exposure of UV-B irradiation to wild rocket

For all UV-B treatments, a UV-B chamber equipped with UV-B lamps (Philips UV Broadband TL 40W/01 RS) with an aluminium reflector realized with vega® UV, a PVD surface specifically developed to optimize the reflectance in the UV bandwidth of 315 nm, was used as shown in Fig. 3, was used. Two separate growing cycles were conducted, and the identical growing conditions were used in both cycles. Each study commenced with a set of 36 pots, which were then divided into three distinct groups following germination. One group represented the control condition, while the other two groups represented two different treatments with various UV-B exposure levels.

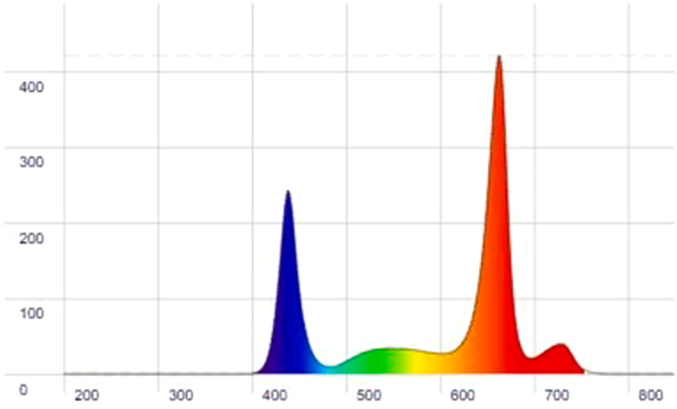

Fig. 2. Spectral quantum distribution used in greenhouse for wild rocket (R : 77.1% ; G+Y :17.9% ; B : 5%).

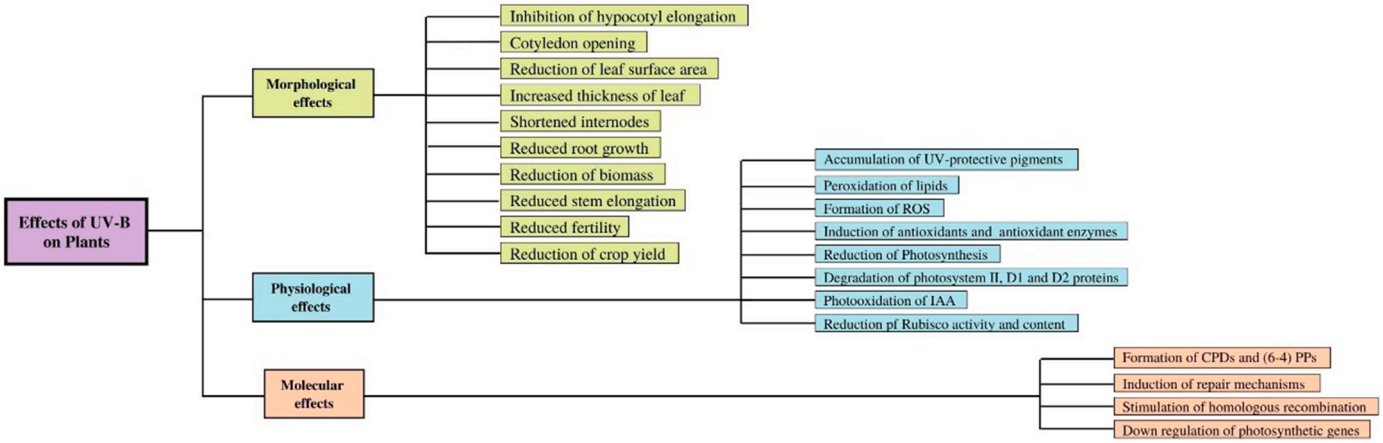

Fig. 1. Effects of UV-B irradiation on morphological, physiological and molecular level in plants.

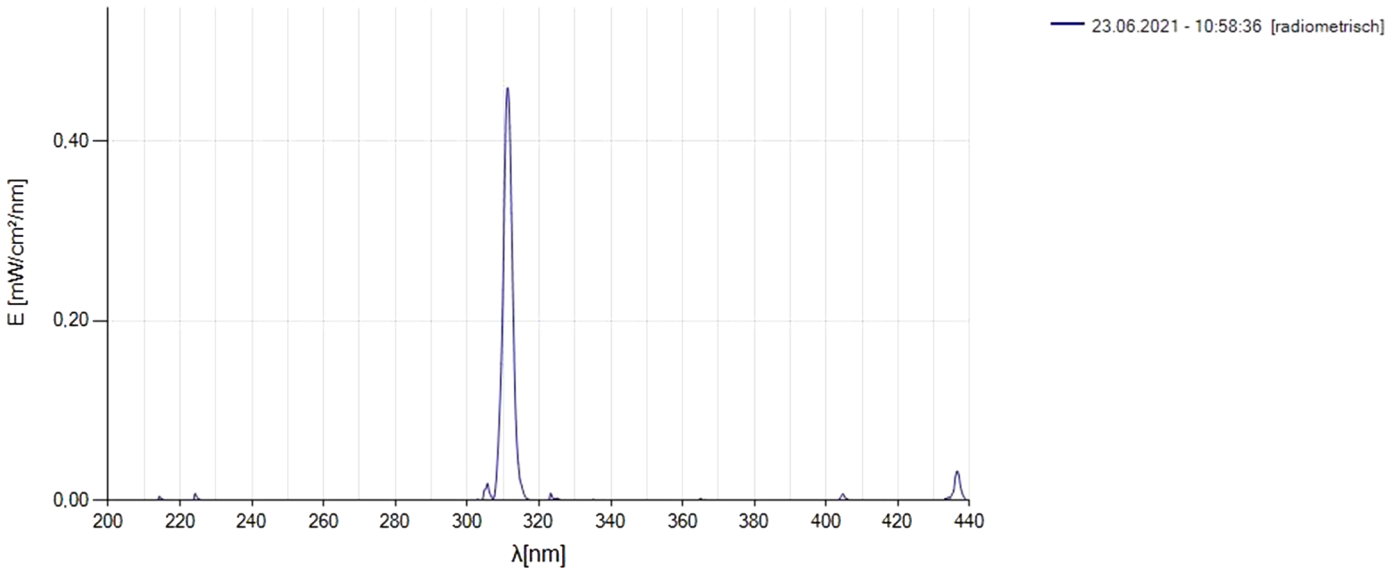

Fig. 3. Spectrum of the UV-B lamps. A single wavelength of 315nm was subjected on the wild rocket plants.

In the first trial, two different treatments were tested, resulting in cumulative energies of 86.4 KJ/m^2 and 172.8 KJ/m^2, respectively. In the second trial the doses were lower to moderate, corresponding to 43.2 KJ/m^2 and 21.6 KJ/m^2 cumulative energies, based on the first trial's findings. The UV-B lamp irradiance was constant (4 W/m^2) and the cumulative UV-B dosage was the result of different time of exposure as shown in Table 1.

When plants were sufficiently developed (10-12 cm, height), the UV-B treatments, once per week, in the first trial were administered overall twice, about two weeks before harvest, on the nights of June 24th and July 1st, 2021, respectively. The exposure time of UV-B stress was 12 and 6 hours per day for doses of 172.8 KJ/m^2 and 86.4 KJ/m^2 respectively (collective UV-B exposure = 24 h for 172.8 KJ/m^2 and 12 h for 86.4 KJ/m^2). The time scheme for the UV-B exposure is represented in Table 2.

The UV-B treatments, however, were administered four times, 6th ,13th, 22nd and 29th April 2022, during the second trial at the lowest doses with the time exposure of 3 hours and 1.5 hours per day for 43.2 KJ/m^2 and 21.6 KJ/m^2 respectively (collective UV-B exposure = 12 h for 43.2 KJ/m^2 and 6 h for 21.6 KJ/m^2), once per week, as shown in Table 3. All treatments have been performed overnight while at dawn the plants were moved back from the UV-B chamber to the glasshouse under the LED lights. Non-destructive tests were performed by using a Multipigment meter and portable fluorimeter, the day after each UV-B treatment to determine the plants' physiological condition. Only measurements obtained right before harvesting, nevertheless, will be displayed for non-destructive results and discussion.

2.3. Total chlorophylls and total carotenoids

Total chlorophylls and carotenoids were extracted from the fresh leaf tissues (around 50 mg) in 5 mL of 99.9% methanol. The samples were

Table 1
Description related to the UV-B light source, studied plant, cumulative UV-B doses in Kilojoule per square meter and the total exposure time of the UV-B treatments.

UV-B light source	Lamp Irradiance (W/m^2)	Cumulative UV-B doses	Total exposure time
Philips UV Broadband TL 40W/01 RS)	4w	172.8 KJ/m^2	24 hours
		86.4 KJ/m^2	12 hours
		43.2 KJ/m^2	12 hours
		21.6 KJ/m^2	6 hours

Table 2
Time scheme of an automatic UV-B treatments of both UV-B 172.8 KJ/m^2 and 86.4 KJ/m^2 intensities to wild rocket. For 172.8 KJ/m^2, treatment started at 20:00 and lasted for 12 hours straight for total of 12 hours UV-B treatment. For 86.4 KJ/m^2, starting time was 20:00 and an exposure of 1h was applied to wild rocket after which there is an off-phase of 1h. UV-B treatment started again at 22:00 for 1h until 23:00 followed by a 1h off-phase and so on until 07:00 for total of 360 min/6h of UV-B treatment.

UV-B 172.8 KJ/m^2			UV-B 86.4 KJ/m^2		
ON	OFF	Minutes	ON	OFF	Minutes
20:00	21:00	60	20:00	21:00	60
21:00	22:00	60	22:00	23:00	60
22:00	23:00	60	00:00	01:00	60
23:00	00:00	60	02:00	03:00	60
00:00	01:00	60	04:00	05:00	60
01:00	02:00	60	06:00	07:00	60
02:00	03:00	60			
03:00	04:00	60			
04:00	05:00	60			
05:00	06:00	60			
06:00	07:00	60			
07:00	08:00	60			
		Total: 12 hours			**Total: 6 hours**

Table 3
Time scheme of an automatic UV-B treatments for UV-B 43.2 KJ/m^2 and 21.6 KJ/m^2 doses to wild rocket. For 43.2 KJ/m^2, treatment started at 20:00 and lasted for 30 minutes after which there was an off-phase for next 1 hour and 30 minutes. Treatment started again at 22:00 for 30 minutes exposure followed by 1 hour and 30 minutes off-phase and so on until 06:30 for total 3 hours of UV-B treatment. Moreover, for 21.6 KJ/m^2 starting time was 20:00 but the exposure was only for 15 minutes after which there was an off phase for 2 hours and so on. The total UV-B treatment for 21.6 KJ/m^2 was only 1 hour and 30 minutes.

UV-B 43.2 KJ/m^2			UV-B 21.6 KJ/m^2		
ON	OFF	Minutes	ON	OFF	Minutes
20:00	20:30	30	20:00	20:15	15
22:00	22:30	30	22:00	22:15	15
00:00	00:30	30	00:00	00:15	15
02:00	02:30	30	02:00	02:15	15
04:00	04:30	30	04:00	04:15	15
06:00	06:30	30	06:00	06:15	15
		Total: 3 hours			**Total: 1 hour 30 minutes**

kept in a dark room at 4 °C for 24 h. Absorbance readings were measured at 665.2 nm and 652.4 nm for chlorophyll pigments and 470 nm for total carotenoids. Chlorophyll and carotenoid concentrations were calculated using Lichtenthaler's formula (Lichtenthaler, 1987).

2.4. Phenolic index and total anthocyanins

For the extraction of the phenolic compounds, around 50 mg of leaves were placed in 5 mL of acidified methanol (1% HCl v/v) and extracted overnight in the dark. The phenolic index was calculated as the absorbance measured at 320 nm. The phenolic index was used as an indication of the total phenolics content. In this method, the total phenols were estimated by measuring absorbance at 320 nm using a UV-Vis spectrophotometer, as previously showed (Ke and Saltveit, 1989). The total anthocyanins were measured from the same extracts. The concentration of anthocyanins was expressed as cyanidin-3-glucoside equivalents and determined spectrophotometrically at 535 nm using the extinction coefficient εM of 29,600 (Ferrante et al. 2004).

2.5. Total glucosinolates determination

Spectrophotometric estimation of total glucosinolates was done with minor modifications (Mawlong et al. 2017). One gram of frozen leaf material was ground using liquid nitrogen, then about 300 milligrams of powder were transferred into 15 mL tubes with 6 mL of 80% methanol. After overnight agitation at room temperature, the samples were centrifuged (ALC centrifuge-model PK130R) at 4000 rpm for 10 min, the pellets were discharged, and the supernatants were filtered through a 0.45 μm syringe filter. Two sets of tubes have been prepared for each sample, a set containing 100 μL of filtered extract, 300 μL of distilled water and 3 mL of reagent (58.8 mg of Na tetrachloropalladate + 100 mL acidic water). The second set of tubes was prepared with 100 μL of filtered extract, 300 μL of distilled water and 3 mL of acidic water. After a vigorous agitation and incubation at room temperature for 1 h, absorbance was measured at 425 nm. The absorbance readings of samples without reagent were subtracted to those with reagents. Total glucosinolates content was calculated based on a standard curve (0-2000 nmol) of glucocheirolin potassium salt.

2.6. Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was measured to estimate the possible oxidative damage of leaves subjected to different UV-B doses. This was assessed by using the thiobarbituric acid reactive substances (TBARS) method (Heath and Packer, 1968). Briefly, one gram of leaf tissue was ground in 5 mL of trichloroacetic acid (TCA) of 0.1% w/v and centrifuged (ALC centrifuge-model PK130R) at 4000 rpm for 10 min. One mL of the extract was mixed with 4 mL of 20% (w/v) TCA, 25 L of 0.5% thiobarbituric acid (TBA), and distilled water. After mixing, the extract was heated at 95 °C for 30 min in a water bath and then cooled in ice. The absorbance at 600 nm was subtracted from the reading at 532 nm (as an index of non-specific turbidity) and the concentration of TBARS were expressed as malondialdehyde (MDA) equivalents (nmol g^{-1} F.W.), with the extinction coefficient $\varepsilon M = 155\ mM^{-1}\ cm^{-1}$.

2.7. Nitrate content

The nitrate content was assessed based on Cataldo's method (Cataldo et al. 1975). Around 1 g of leaves was ground with 4 mL of distilled water. The extract was centrifuged (ALC centrifuge-model PK130R) at 4000 rpm for 15 min and the supernatant was recovered and used for the colorimetric determination of nitrate and sugars.

Twenty μL of the sample was added to 80 μL of 5% salicylic acid in sulphuric acid (H_2SO_4) and to 3 mL of sodium hydroxide (NaOH) 1.5 N. The samples were cooled at room temperature and the spectrophotometric readings were done at 410 nm. The nitrate content was estimated based on a potassium nitrate (KNO_3) standard calibration curve (0-10 mM).

2.8. Total sugars, reducing sugars and sucrose content

The extracts used were the same previously prepared for the nitrate content. The sucrose assay was performed by mixing 0.2mL of leaf extract with 0.2mL of 2 M NaOH and incubated in a water bath at 100 °C for 10 min. After which 1.5 mL hot resorcinol buffer (containing 30% hydrochloric acid, 1.2mM resorcinol, 4.1mM thiourea 1.5M acetic acid) was added to samples and incubated in a Dubnoff bath (PID) at 80∘C for another 10 min. After cooling at room temperature, the O.D. was determined at 500 nm and a sucrose standard curve (0-2 mM) was used for calculating the final concentration (Rorem et al. 1960).

Reducing sugars were determined on 0.2 mL of extract, that was added to 0.2 mL of a solution containing 62.6 mM dinitrosalicylic acid (DNS) and 1.52 M potassium sodium tartrate (Miller, 1959). The reaction mixture was heated at 100 °C for 5min, then 1.5 mL of distilled water was added, and absorbance was measured at 530 nm. The reducing sugars were expressed as glucose equivalent and calculated using a glucose standard curve (0-4 mM).

The total sugars concentration was assessed spectrophotometrically following the anthrone method (Yemm and Willis, 1954) with slight modifications. The anthrone reagent (10.3 mM) was prepared dissolving anthrone in ice-cold 95% H_2SO_4. The then, 0.5 mL of extract was placed on top of 2.5 mL of anthrone reagent and kept in ice for 5 min. after that, the mix was vortexed vigorously, heated at 95 °C for 10 min and left to cool in ice. Readings were performed at 620 nm and total sugars concentration was calculated, based on a glucose calibration curve (0-4 mM).

All spectrophotometric determinations have been performed using the Evolution 300 UV-Vis spectrophotometer (Thermo Scientific).

2.9. Non-destructive estimation using MPM-100 Multipigment meter

After each UV-B application, as well as at harvest, Nitrogen-Flavonol Index, chlorophyll, flavonols and anthocyanin contents were measured non-destructive using MPM-100 Multipigment meter (ADC BioScientific Ltd, UK) on fully expanded leaves (Cerovic et al. 2008). However, the later three parameters were considered while explaining the UV-B effect on the wild rocket.

2.10. Non-destructive estimation using fluorimeter

This analysis has been performed during the second trial in order to measure the leaves light utilization and health status of photosystem II. The chlorophyll *a* fluorescence was estimated on dark adapted (30 min) wild rocket leaves using a portable fluorimeter (Handy PEA; Hanstech, Kings Lynn, UK). The parameters measured were the maximum quantum efficiency pf photosystem II (Fv/Fm) and the performance index (PI), which provides information about the relative leaf functionality. The PI includes three independent parameters: the intensity of active reaction centres (RCs), the efficiency of electron transport and the probability that an absorbed photon will be trapped by the RCs.

2.11. Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test. Analyses were performed using GraphPad Prism version 6 for Windows (GraphPad Software; La Jolla, California, USA, www.graphpad.com).

3. Results

3.1. Physiological and quality evaluation of wild rocket for the UV-B doses of 86.4 KJ/m^2 and 172.8 KJ/m^2

Referring to the (Fig. 4a), different UV-B doses resulted in acquiring different amounts of chlorophyll a and b in the preliminary results on wild rocket, subjected to higher UV-B doses of 172.8 KJ/m^2 and 86.4 KJ/m^2. An increase of chlorophyll content (1.058 μg/mg) in the UV-B treatment of 172.8 KJ/m^2 compared to the (0.7655 μg/mg) and (1.012 μg/mg) of 86.4 KJ/m^2 and control was recorded. Despite showing slight variations, no significant differences have been recorded overall in the destructive analysis of chlorophyll content. Moreover, a similar trend has been observed for the carotenoids content in which 172.8 KJ/m^2 UV-B dose exceeded in the accumulation of carotenoid pigment (0.1753 μg/mg) against the (0.0792 μg/mg) and (0.1590 μg/mg) for the 86.4 KJ/m^2 and the control respectively. Although non-significant difference prevailed between the control and UV-B treatment of 172.8 KJ/m^2, these two treatments however, were significantly different to the UV-B 86.4 KJ/m^2 for total carotenoid accumulations (Fig. 4b).

Considering the secondary metabolism, a slight decline was noticed in the phenolic index of the two UV-B treatments compared to the control. No significant variations have been recorded between the treatments including control in which against (25.58 ABS_{320} g^{-1} FW) phenolic index of the control, the UV-B dose of 86.4 KJ/m^2 accumulated (24.48 ABS_{320} g^{-1} FW) and UV-B dose of 172.8 KJ/ m^2 (22.03 ABS_{320} g^{-1} FW) phenolic index respectively (Fig. 4c). Likewise phenolic index, non-significant differences were evident for anthocyanins among both UV-B treatments and the control. A slight increase, however, was recorded in the anthocyanin concentration of 172.8 KJ/m^2 UV-B with a value (20.10 Cyanidin eq. mg 100 g^{-1} FW) against the (16.96 Cyanidin eq. mg 100 g^{-1} FW) and (18.81 Cyanidin eq. mg 100 g^{-1} FW) of 86.4 KJ/m^2 and control respectively (Fig. 4d).

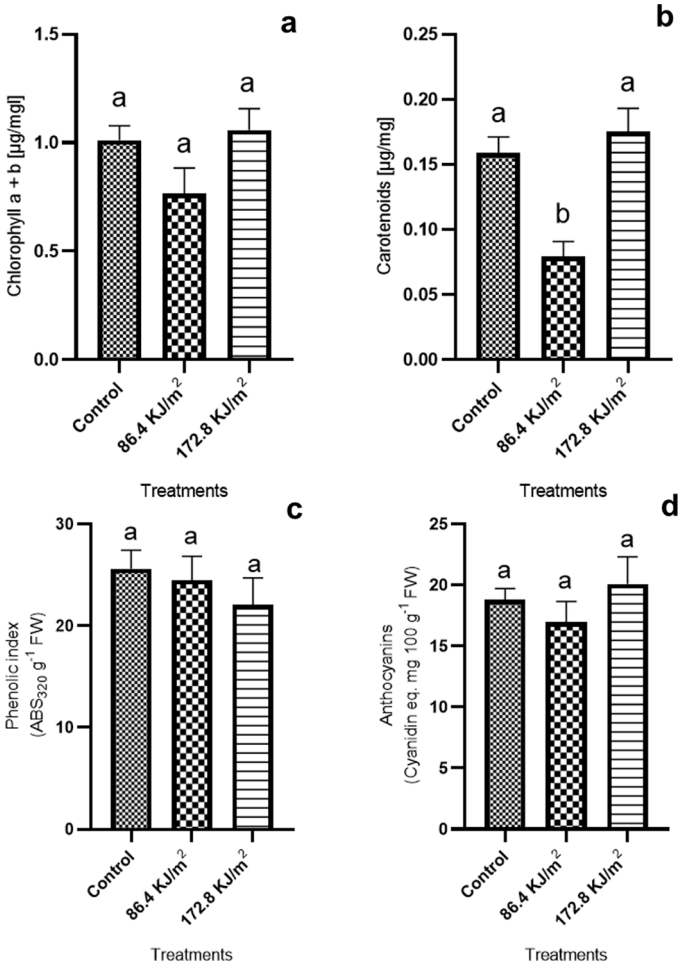

Fig. 4. (a) Chlorophyll (b) Carotenoids (c) Phenolic Index (d) Anthocyanins contents of wild rocket treated with 86.4 KJ/m^2 and 172.8 KJ/ m^2 UV-B doses. Values are mean (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

According to (Fig. 5a), a significant increase for the nitrate contents among all the treatments was observed with the increase in the UV-B dose. Nitrates in control wild rocket plants for this experiment were recorded at (662 mg/Kg) which increased successively to (1496 mg/Kg) and (2707 mg/Kg) in UV-B doses successively. Possibly, this increase in the nitrates and decrease in the nutritional quality of wild rocket is due to an impairment in the N metabolism under high level of UV stress. However, considering both UV-B doses of 86.4KJ/m^2 and 172.8KJ/m^2 with respect to the control, non-significant differences were found for glucosinolates among all the utilized treatments, with control depicting (76.65 mg/100g) of glucosinolates to (69.27 mg/100g) and (73.54 mg/100g) of glucosinolates for the respective UV-B treatments of 86.4KJ/m^2 and 172.8 KJ/m^2 (Fig. 5b).

A decrease in total sugar contents was seen in both UV-B doses of 86.4 KJ/m^2 and 172.8 KJ/m^2 compared to the control in which significantly higher total sugar value was recorded at (6.505 mg Glu eq g^{-1} FW) compared to the ones recorded for UV-B induced wild rocket plants such as (4.368 mg Glu eq g-1 FW) and (3.155 mg Glu eq g-1 FW) for both 86.4 KJ/m^2 and 172.8 KJ/m^2 respectively. However, non-significant differences were found in the total sugar contents between the two UV-B treatments in this experiment (Fig. 5c). Similarly, same trend has been observed for the reducing sugars, with a continuous decline in reducing sugar contents for the successive increase in the UV-B doses (Fig. 5d). A strong significant difference has been observed between all the three treatments with control being the highest 7.235mg/Kg, 86.4 KJ/m^2 being the next with 5.747 mg/Kg and 4.225 mg/Kg for the 172.8 KJ/m^2 UV-B treatments. However, an increasing trend was observed while investigating the sucrose accumulation in the wild rocket. There was a significant difference between the control (0.4771 mg/g) and the higher UV-B dose of 172.8 KJ/m^2 in which highest sucrose accumulation was observed at (0.8108 mg/g). Furthermore, the UV-B dose of 86.4 KJ/m^2 was intermediate between aforementioned treatments of control and 172.8 KJ/m^2 with an observed sucrose level of (0.7606 mg/g) (Fig. 5e).

3.2. Physiological and quality evaluation of wild rocket for 21.6 KJ/m^2 and 43.2 KJ/m^2 UV-B doses

With lower UV-B doses of 21.6 KJ/m^2 and 43.2 KJ/m^2 (Fig. 6a), non-significant differences were recorded in the chlorophyll content between the control and the applied UV-B doses, a finding consistent with the one which used UV-B doses of 86.4 KJ/m^2 and 172.8 KJ/m^2, respectively. With the observed value of (0.8596 μg /mg) compared to (0.6952 μg /mg) and (0.6613 μg /mg) of the control and 43.2 KJ/m^2 UV-B dose, respectively, a slight increase in the chlorophyll content of 21.6 KJ/m^2 was seen. However, compared to the control, carotenoids in the UV-B treatments of 21.6 KJ/m^2 and 43.2 KJ/m^2 showed a progressive non-significant decreasing trend (Fig. 6b). With control holding the higher accumulation of carotenoids at 0.0834 μg/mg, a decline at 0.0651 μg/mg for 21.6 KJ/m^2 and 0.0523 μg/mg for 43.2 KJ/m^2 was recorded for wild rocket.

Interestingly, as seen from the (Fig. 6c) the plants under UV-B dose of 21.6 KJ/m^2 demonstrated higher significant phenolic index (28.43 ABS_{320} g^{-1} FW) compared to the control (22.86 ABS_{320} g^{-1} FW) and 43.2 KJ/m^2 UV-B dose (21.54 ABS_{320} g^{-1} FW), which were non-significant to one another. The results of the high phenolic index of 21.6 KJ/m^2 UV-B dose is quite promising, if compared with intense UV-B doses. In terms of anthocyanins production, (Fig. 6d) the UV-B dose of 21.6 KJ/m^2 (22.46

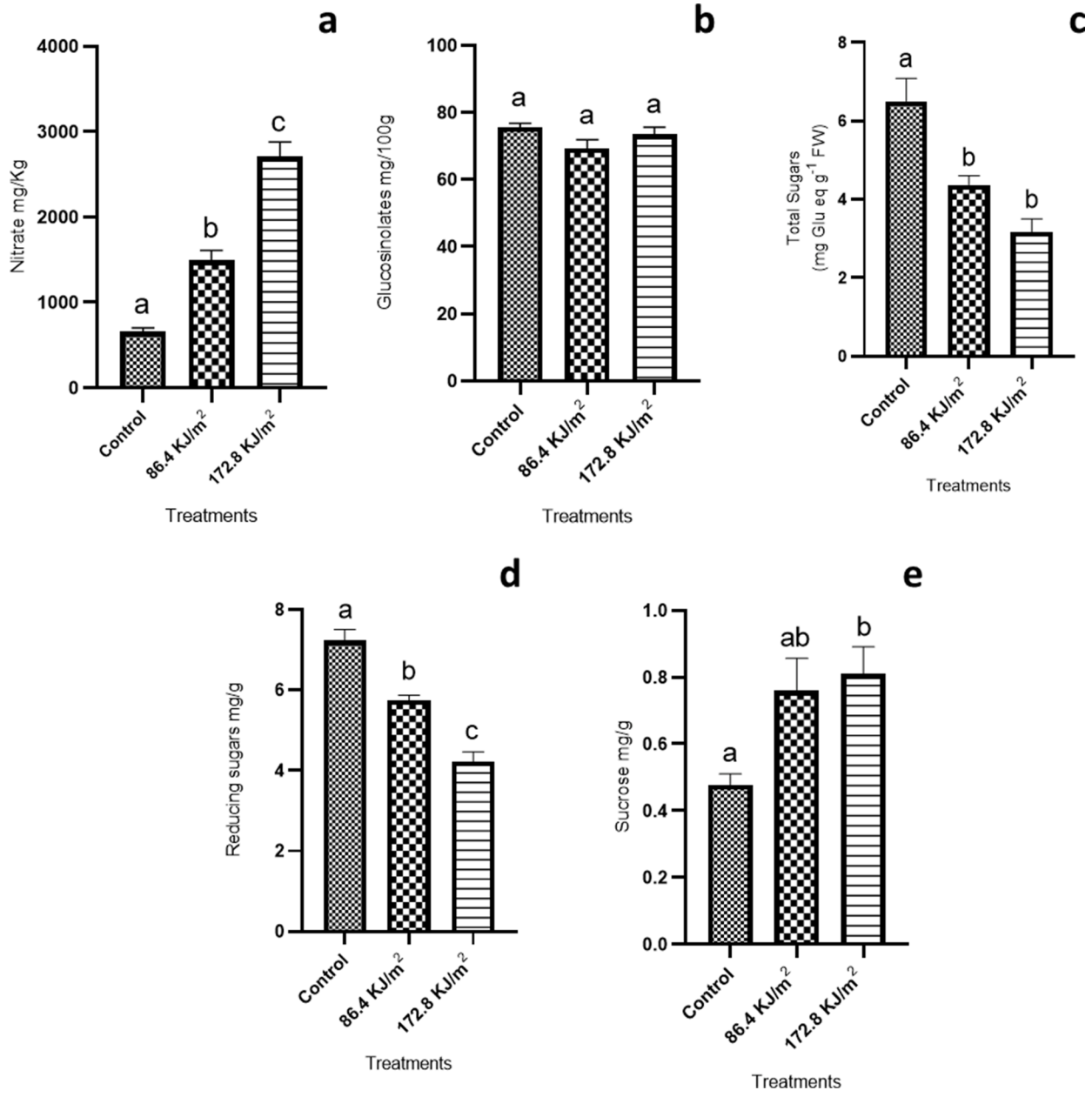

Fig. 5. (a) Nitrates (b) Glucosinolates (c) Total sugars, (d) Reducing sugars, (e) Sucrose contents of wild rocket treated with 86.4 KJ/m^2 and 172.8 KJ/ m^2 UV-B doses. Values are mean (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

Cyanidin eq. mg 100 g^{-1} FW) surpassed the other two treatments of control and 43.2 KJ/m^2 which hold (18.60 Cyanidin eq. mg 100 g^{-1} FW) and (20.56 Cyanidin eq. mg 100 g^{-1} FW) respectively. However, this trend diverges significantly from the trial performed previously with higher UV-B doses of 86.4 KJ/m^2 and 172.8 KJ/m^2, in which no appreciable significant differences between the control and UV-B treatments were observed.

Referring to (Fig. 7a), non-significant higher accumulation in the nitrate contents was observed in both UV-B treatments of 21.6 KJ/m^2 (1322 mg/Kg) as well as 43.2 KJ/m^2 (892.2 mg/Kg) with respect to control (767 mg/Kg), which is remarkably comparable to earlier UV-B experiment with higher UV-B doses. Increased significant differences have been noticed between the control (67.33 mg/100g) and the UV-B 43.2 KJ/m^2 (72.73 mg/100g) while the 21.6 KJ/m^2 (70.31 mg/100g) was non-significant to the control (Fig. 7b). These results however, differ from the previous UV-B experiment, in which higher UV-B doses did not yield an increased glucosinolates compared to the control. To evaluate the wild rocket's lipid peroxidation, TBARS assay was performed (Fig. 7c). Higher UV-B at 43.2 KJ/m^2 was significantly found to damage membranes compared to lower UV-B at 21.6 KJ/m^2. When compared to the (4.724 nmol MDA g^{-1} FW) and (4.269 nmol MDA g^{-1} FW) values of control and 21.6 KJ/m^2, malondialdehyde measurements showed a higher value of (6.758 nmol MDA g^{-1} FW) in 43.2 KJ/m^2 UV-B treatment respectively.

Both UV-B at 21.6 KJ/m^2 (1.443 mg Glu eq g^{-1} FW) and 43.2 KJ/m^2 (1.778 mg Glu eq g^{-1} FW) showed higher total sugar accumulations than the control (1.778 mg Glu eq g^{-1} FW). However, only 43.2 KJ/m^2 UV-B treatment yielded significant difference in total sugars accumulations compared to both 21.6 KJ/m^2 and the control (Fig. 7d). Non-significant differences in the reducing sugars were recorded among all the treatments where 43.2 KJ/m^2 UV-B dose accumulated (6.70 mg/g) of reducing sugars with respect to (5.990 mg/g) and (5.927 mg/g) in 21.6 KJ/m^2 UV-B and control respectively (Fig. 7e). This is quite different from what was observed in the higher UV-B treatments where UV-B

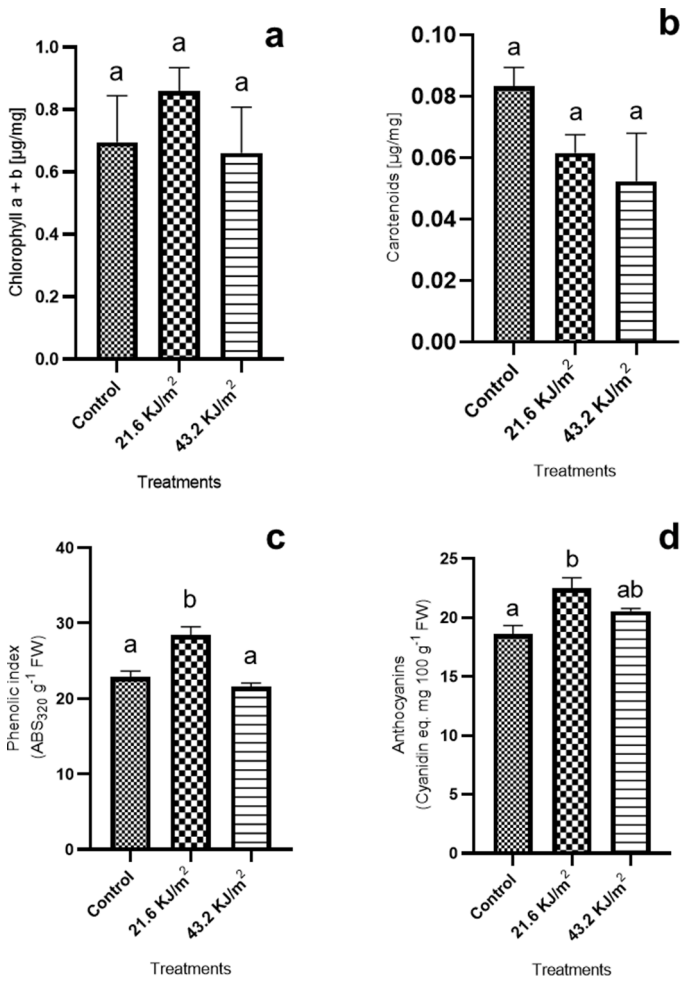

Fig. 6. (a) Chlorophyll a and b, (b) Carotenoids (c) Phenolic Index (d) Anthocyanins contents of wild rocket treated with 21.6 KJ/m^2 and 43.2 KJ/ m^2 UV-B doses. Values are mean (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

treated wild rocket accumulated fewer reducing sugars than the control. Non-significant differences were recorded among all the treatments for sucrose where in contrast to control (0.7453 mg/g), a modest increase in sucrose accumulation of (0.9808 mg/g) in 21.6 KJ/m^2 and (0.9056 mg/g) in 43.2 KJ/m^2 was observed.

3.3. Non-destructive estimation of chlorophyll, flavanols and anthocyanins by using MPM-100 multi-pigment meter

Non-destructive estimation of chlorophyll, flavanols and anthocyanins have been carried out with the help of MPM-100. A decrease in chlorophyll has been estimated for all the UV-B treatments while the two UV-B treatments of 86.4 KJ/m^2 and 21.6 KJ/m^2 showed a significant reduction in chlorophyll estimated values compared to the control as represented in (Fig. 8 a,d). Flavanols were enhanced in all the UV-B treatments in which a significant enhancement was observed for 21.6 KJ/m^2 and 43.2 KJ/m^2 while the UV-B 86.4 KJ/m^2 and 172.8 KJ/m^2 showed an increased non-significant accumulation of flavanols compared to the control (Fig. 8b & e). Likewise, flavanols, marked increase in the estimation of anthocyanins was recorded in the UV-B treatments, out of which only 172.8 KJ/m^2 showed the significant increment compared to the control (Fig. 8c & f).

3.4. Non-destructive estimation of maximum quantum efficiency of photosystem II (Fv/Fm) and performance index (PI)

By using Handy PEA+ fluorimeter, a non-destructive estimation of maximum quantum efficiency of photosystem II as well as the overall leaf performance index was evaluated. UV-B has shown an obvious stress effect on the overall leaf performance index of wild rocket, with a significant decrease in 43.2 KJ/m^2 UV-B compared to the control. However, with less intense UV-B of 21.6 KJ/m^2, the performance index was found intermediate between the control and intense UV-B treatment of 43.2 KJ/m^2. Moreover, it was noted that the photochemical efficiency of photosystem II decreased significantly in both 43.2 KJ/m^2 and 21.6 KJ/m^2 UV-B treatment compared to the control (Fig. 9b).

4. Discussion

Ultraviolet radiation regulates plant development and secondary metabolism while being an environmental stressor (Jaiswal et al. 2022). Applying low and ecologically relevant UV-B to plants can induce the accumulation of phenolic compounds, carotenoids, and glucosinolates, which leads to the biofortification of horticulture crops with nutraceuticals. In light of this, ultraviolet irradiation technology may be a quick and efficient method to treat fruits, vegetables and salad for daily consumption of healthy foods. Moreover, numerous epidemiological studies have proven an inverse relationship between consumption of green foods and the occurrence of cardiovascular and cancerous disorders (Ağagündüz et al. 2022). This theme was kept in mind while subjecting the rocket to the various UV-B doses for the accumulation of these essential secondary metabolites.

4.1. Non-destructive and destructive quality evaluation of wild rocket for accumulation of secondary metabolites

In the current study, which used a range of UV-B doses, it was discovered that *Chl a* and *b* levels decreased at doses of 86.4 KJ/m^2 and 43.2 KJ/m^2 while the trend was otherwise for the remaining two treatments. The same results were recorded for the non-destructive estimation of chlorophyll using MPM-100 multi-pigment meter. Chlorophyll aids plants in absorbing excess light, turning it into photosynthetic electron transport and hence ensuring the likelihood of photosynthesis. The damage caused by UV-B to the chloroplast followed by the degeneration of photosystem II (Salama et al. 2011) can be the explanation for the loss of chlorophyll in this study with higher 86.4 KJ/m^2 and 43.2 KJ/m^2 UV-B doses. According to Szatatelman et al. (2015) high UV-B exposures are major abiotic stresses that can damage chlorophyll and cause *Arabidopsis thaliana* to produce senescence-related genes. UV-B may have an indirect influence (decrease in *Chl a* and *b* levels or leaf area) or a direct effect (damage to photosystems) on 3–90% of photosynthesis in various plants (Surabhi et al. 2009). Additionally, Quan et al. (2018) evaluated the drop in photosynthesis and chlorophyll content and reported loss in the leaf and stem biomass after subjecting *Scutellaria baicalensis* to UV-B radiation. Research conducted by Xie et al. (2022), revealed a 20% decrease in *Chl b* in common duckweed (*Lemna minor)* when exposed to UV-B however, no significant effect was seen for *Chl a*. However, with UV-B dose of 21.6 KJ/m^2, it was observed that the chlorophyll content exhibited a favorable increase as compared to control. Referring to the earlier findings of Helsper et al. (2003), who also noted that UV radiation, in particular UV-A, caused a minimal increment in *Chl a* and *b* in the leaves of *Rosa hybrida* and *Fuchsia hybrida*. It is noteworthy that there is not enough literature to support and explain the increase or no effect of UV-B for chlorophyll contents. Future trials must conduct a thorough investigation of irradiance intensity and time exposure to explain these variations as UV-B radiation effects are likely complex, revealing surprising differences in how plants respond to different doses.

Carotenoids are present in a variety of photosynthesizing species,

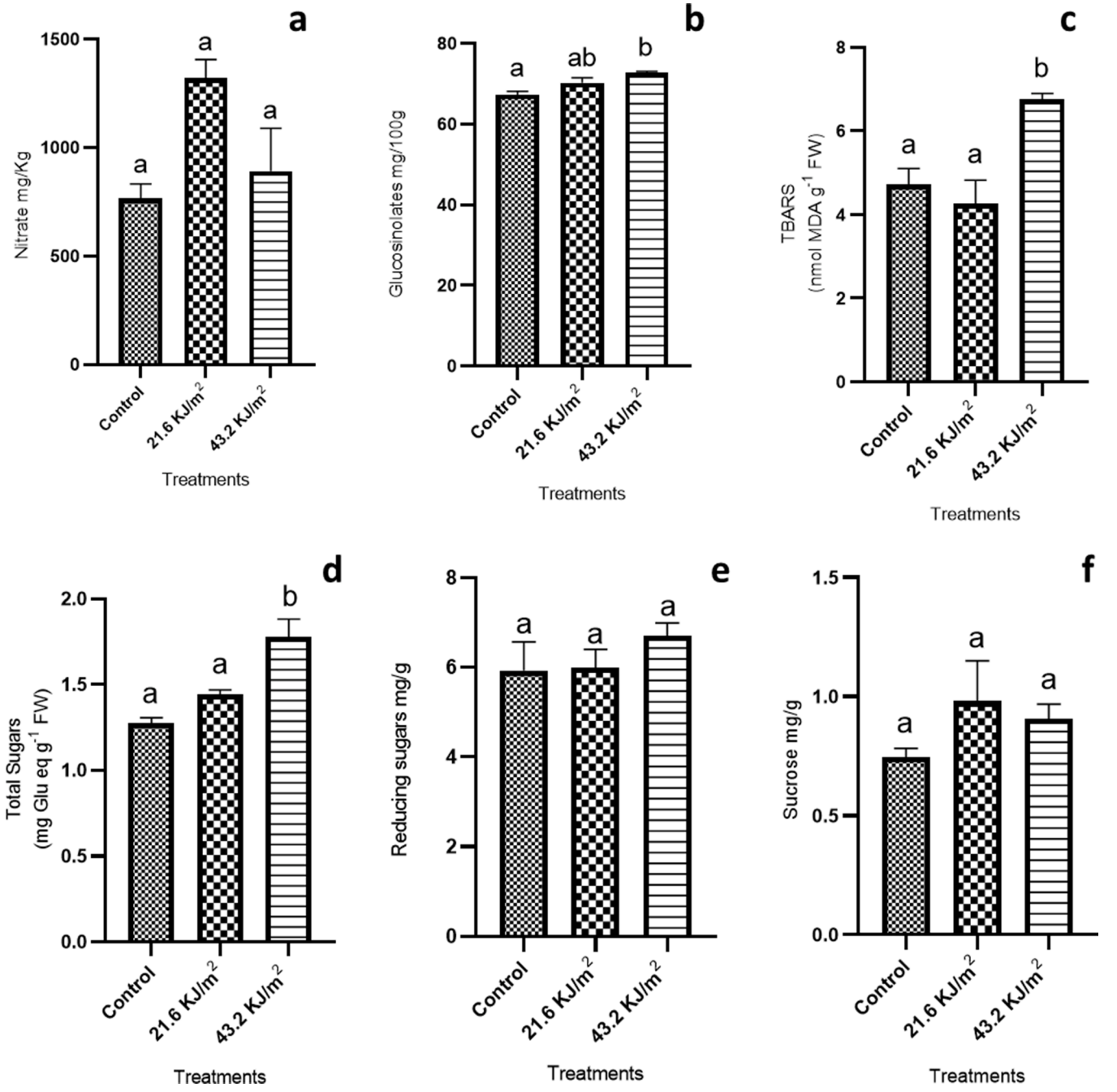

Fig. 7. (a) Nitrates, (b) Glucosinolates (c) TBARS (d) Total sugars (e) Reducing sugars (f) Sucrose contents of wild rocket treated with 21.6 KJ/m^2 and 43.2 KJ/ m^2 UV-B doses. Values are mean (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

including plants, fungi, algae, and bacteria. They are subdivided into two main subgroups, carotenes and xanthophylls, essential for the process of harnessing light (carotenes), removing reactive oxygen species, and shielding the chlorophyll (xanthophylls) from photooxidation. In the current study, a decrease has been observed in the carotenoid's biosynthesis compared to control for the three UV-B treatments except the acute exposure of UV-B 172.8 KJ/m^2. A similar increase was noted by (Hector et al 2007), who found that genes involved in the production of carotenoids are typically down-regulated following chronic UV-B exposures but are up-regulated following acute UV-B exposure. However, under UV-B irradiation, the precise regulatory functions of the genes involved in carotenoids metabolism remained unknown. Carotenoids accumulation is controlled by related transcription factors, phytohormones, and abiotic stress, as well as the expression of numerous important pathway genes, including *PSY, LCY-E, LCY-b, CHY-b*, and *VDE* (Toledo-Ortiz et al. 2010). Research conducted on peach for continuous 48-hour of acute UV-B exposure has revealed that the lutein-producing Lycopene epsilon cyclase (LCY-E) gene enhanced lutein generation by up to 80% however, no changes were experienced for phytoene synthase (PSY) gene (Jaiswal et al. 2021). Several other studies found that increasing carotenoids production such as lutein protected the photosystem II from long-term UV-B stress in wheat endosperm (Yu et al. 2022). Additionally, red cabbage sprouts, maize kernels, and bell peppers all experienced an increase in total carotenoids when exposed to UV-B stress (Martinez-Zamora et al. 2021). Further investigation, however, is required to understand why the carotenoids experienced a downfall in this experiment for the less strong UV-B treatments compared to the acute 172.8 KJ/m^2.

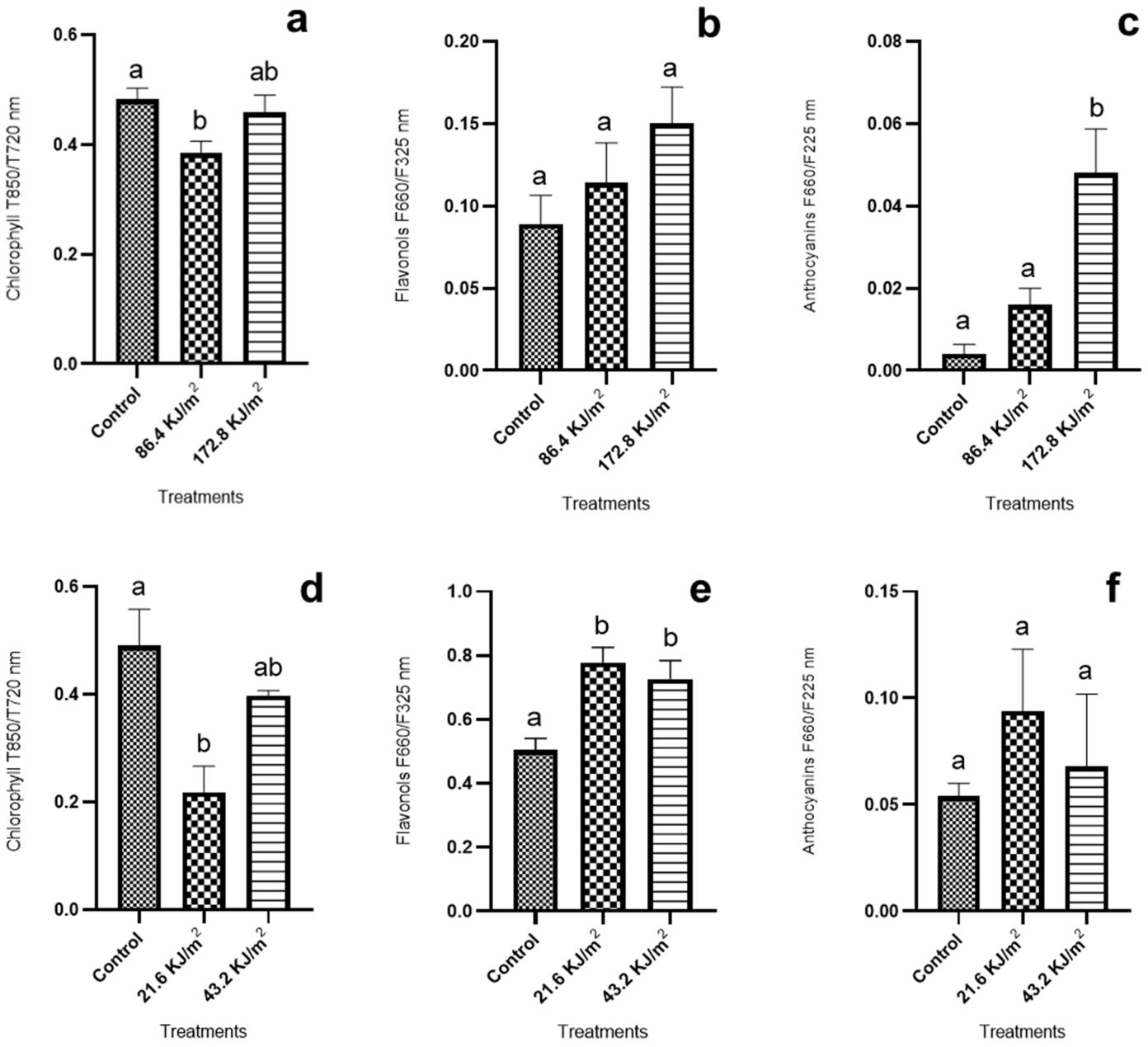

Fig. 8. Non-destructive estimation of chlorophyll, flavanols and anthocyanins for the control and UV-B doses of 86.4 KJ/m^2 and 172.8 KJ/m^2 are shown in (a), (b) and (c). while the estimation for the same parameters for the control and UV-B doses 21.6 KJ/m^2 and 43.2 KJ/m^2 are shown in (d), (e) and (f). Values are means (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

Phenolic compounds can be induced in plants and are often thought of as phytoalexins with redox capabilities that can help plants withstand a variety of environmental challenges. In the current research, the 21.6 KJ/m^2 UV-B treatment showed a significant increase in the phenolic index and anthocyanins with less deleterious effects, which is consistent with scientific findings of UV-B stress in radish sprouts, apple (*Malus domestica*), turnip (*Brassica rapa*), *Triticum* sprouts, swiss chard (*Betula pendula)* and *Arabidopsis thaliana* (Su et al. 2016). Moreover, during *non-destructive* analysis, peaks have also been observed for the flavanols as well as anthocyanins estimation, an effect that has been widely studied (Bhatt et al. 2022). It is now established that UV-B exposure to plants enhances the accumulation of secondary metabolites, such as phenolics and anthocyanins, as well as aids in photomorphogenesis. As a matter of fact, during the UV response, the UV-responsive transcription factors *HY5* and *PFG1/MYB12* control the expression of the phenolic and flavonoid genes, such as chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavonol synthase (*FLS*), dihydroflavonol 4-reductase (*DFR*), phenylalanine ammonia lyase. The phenolic compounds were higher in pigeon pea root cultures of 2 to 4h of UV-B stress, followed by a gradual decline in the corresponding time span up to 24 h, which was likely to be related to the regulation competition in metabolic flow dominated by CHS and STS, but the accumulation of phenolic compounds, especially cajaninstilbene, was observed to be increase up to 2.31 folds compared to the control (Gai et al. 2022). When boosting the production of phytochemicals in a plant industry using artificial UV-B lights, consideration should be made to both UV irradiation dose and wavelength. This idea was proposed by (Lee et al. 2022) while examining the response of canola (Brassica napus) in a plant factory with artificial light. Numerous studies on a variety of fruits (Ortega-Hernandez et al. 2020) and vegetables (Formica-Oliveira et al. 2017) have led researchers to the conclusion that not all phenolic compounds are equally generated under UV-B stress, although flavonoids and flavonoid glycosides are typically

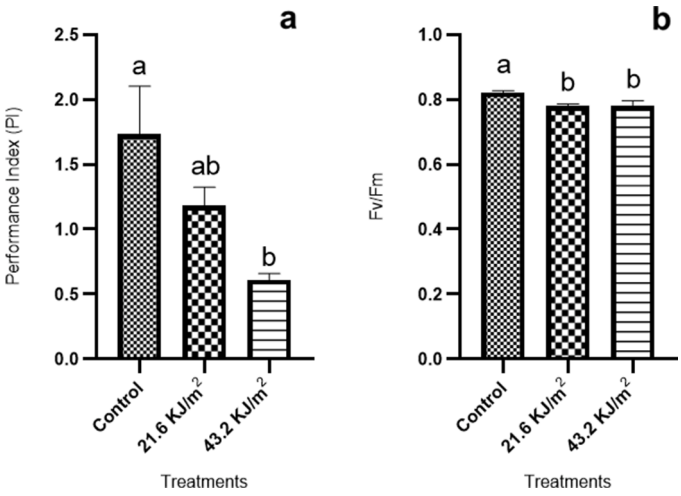

Fig. 9. Non-destructive measurements (a) Maximum quantum efficiency pf photosystem II (*Fv/Fm*) (b) performance index of wild rocket treated with 21.6 KJ/m^2 and 43.2 KJ/m^2 UV-B doses, by using fluorimeter. Values are means (n=5 ± S.E.). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p<0.05$).

more sensitive to UV-B than phenolic acids. The 21.6 KJ/m^2 UV-B dose in our study produced considerable antioxidants compared with the other doses, explained the fact that the different UV-B doses produced different degrees of phenolics and anthocyanins. However, as was previously mentioned, both UV dose and wavelength play a critical role in encouraging the accumulation of essential secondary metabolites in plants.

Glucosinolates are well-known secondary metabolites that contain molecules with sulfur and nitrogen. There are currently 130 glucosinolates known, and they fall into three categories: aliphatic, indolic, and benzoic glucosinolates. According to our findings, the wild rocket contained significantly more glucosinolates after being exposed to UV-B levels of 21.6 KJ/m^2 and 43.2 KJ/m^2 compared to the control. The strong UV-B doses of 86.4 KJ/m^2 and 172. 8 KJ/m^2 did not, however, show any apparent accumulation which probably be disrupted due to the deleterious disturbances in plant overall defense mechanism caused by the intense UV-B doses. Previously, it was reported that Arabidopsis thaliana's glucosinolate levels significantly increased when subjected to UV-B radiation of 5.5 KJ/m^2 dose and had a major impact on the gene's expression responsible for producing Indole glucosinolates (Demkura and Ballare, 2012). Rybarczyk-Plonska et al. (2016) irradiated *Brassica oleracea* L. with 20 KJ/m^2 of UV-B and observed an increase in the production of indole glucosinolates and total aliphatic glucosinolates, in accordance with the UV-B dose of 21.6 KJ/m^2 in this research. The total amount of glucosinolates in broccoli sprouts has been proven to increase at UV-B doses as low as 0.3 to 0.6 KJ/m^2 (Perez-Balibrea et al. 2010). Furthermore, an astounding increase of 148% in glucosinolates was noticed in broccoli sprouts when exposed to 7.16 W/m^2, however, prolonging this exposure to 12 hours for 1.5 W/m^2, a decline in overall glucosinolates has been recorded in Arabidopsis thaliana (Moreira-Rodriguez et al. 2017).

For growth and development, nitrate is the preferred nitrogen source for plants and once it is accomplished, the nitrates along with sugars are used in secondary metabolism. Nitrate uptake, transportation, and dissemination in plants are all facilitated by nitrate transporters (*NRT*). In the current research, it was found that the nitrate content of all UV-B treatments increased following the UV-B exposure. These results are consistent with those in which UV-B stress increased nitrate reallocation in the leaves and roots of Arabidopsis (Wang et al. 2022). Previous studies demonstrated that *NRT1.8* expression was increased to support plants under extreme soil stresses and is recently revealed to be implicated in increased nitrate buildup in shoots by 14.49 times. In addition, further details were made available to explain that UV-B promoted the expression of *ERF1B, ORA59, ERF104,* and *NRT1.8* in Arabidopsis as well as the reallocation of nitrate from hypocotyls to leaves and roots (Zhang et al. 2014). According to our research, the buildup of nitrates in the leaves of wild rocket plants increased significantly after UV-B stresses of 21.6 KJ/m^2, 43.2 KJ/m^2, 86.4 KJ/m^2, and 172.8 KJ/m^2 which give a clue of nitrate storage in leaf for proper functioning of leaves for normal growth and activities of plants. Multiple studies reported that when exposed to UV-B damage, plants store nitrates in their leaves for proper leaf functioning before allocating them to storage organs like tubers (Tegeder and Masclaux-Daubresse, 2018) and rhizomes (Jaiswal et al. 2022). The reverse tendency of nitrate accumulation in corn (*Zea mays*) leaves and roots following the UV-B stress was, however, seen by (Quaggiotti et al. 2004) in which a drastic decrease in the nitrate reductase (NR) activity was noticed in the second leaf stage of corn compared to the control. The nitrate contents of leaves and roots were not altered in another research findings, but it was claimed that UV-B exposure caused some changes to the nitrate metabolism in barley (*Hordeum vulgare*) (Ghisi et al. 2002).

TBARS (Thiobarbituric Acid Reactive Substances) assay is a method used to measure the lipid peroxidation of the membranes in plant cells. These substances such as malonaldehyde (MDA) are formed as a byproduct of lipid peroxidation process and help the researchers to assess the damage caused by the particular stress. This TBARS analysis was conducted for UV-B doses of 21.6 KJ/m^2 and 43.2 KJ/m^2 while keeping in mind that the UV-B-irradiated rocket will clearly suffer damage from the earlier tests with intense UV-B of 86.4 KJ/m^2 and 172.8 KJ/m^2. An intense membrane damage has been observed by the high UV-B dose of 43.2 KJ/m^2, while it was less severe in plants exposed to the lower UV-B dose of 21.6 KJ/m^2. Righini et al. (2019) observed that maize (Zea mays) exposed to UV-B are consistent with the ability of flavones synthase (*FS I and II*) to engage with the lipid membranes, changing their physical properties, and subsequently shielding membranes from the oxidant molecules under the UV-B stress. When exposed to UV-B and high intense light (HIL), Arabidopsis *hy4* mutant sustained more oxidative stress than the wild type (Kreslavski et al. 2021). Additionally, an increase in TBARS of 76% was observed after 90 minutes of UV-B stress, which decreased to 35% at the same time under UV-B + melatonin treatment, emphasizing the melatonin's alleviating effects. Likewise, in an experiment where wheat seedlings were exposed to UV-B exposure (Tian et al. 2007), TBARS increased up to 3.65% compared to the non-treated wheat seedlings. Moreover, a rise of 18% in the levels of lipid peroxidation was documented in the leaves of Micro-Tom tomato by (Mannucci et al. 2020) after treating it with UV-B irradiation, while the roots showed no increment of lipid peroxidation. However, on the 15th day, this increment in TBARS of Micro-Tom leaves ultimately decreased and reached parity with the control.

Sugars have an impact on all stages of the plant life cycle due to their roles as energy and carbon sources and their regulatory functions. They also interact with other signaling molecules, including phytohormones, and regulate the growth and development of plants. They are also key elements of the plant defense mechanism against both biotic and abiotic stressors. In the experiment with UV-B doses of 21.6 KJ/m^2 and 43.2 KJ/m^2, it was observed that the plant responded to the UV-B stress by increasing the total sugars, reducing sugars as well as sucrose contents in a manner previously reported by (Wang et al. 2018), where UV-B dosage of 1.4 KJ/m^{2}d^1 have potentially triggered the expression of sugar transporter genes and aided the accumulation of 1.5 times more total sugars in UV-B treated *Prunus persica* fruits. In another experiment, blueberries were irradiated with low to medium UV-B levels which resulted in an increased sugar buildup without impairing the quality of the fruit (Li et al. 2021). Mariz-Ponte et al. (2021) noticed an increase in the total sugar contents of tomato (*Solanum lycopersicum*) after 30 days of UV-B irradiation, but also formulated that starch levels fluctuated over time under both UV-A and UV-B stress. An upregulation of anthocyanins as well as total sugar accumulation in a UV-B exposed grape

berries were also previously monitored (Martinez-Luscher et al. 2014). Similarly, under a UV-B dose of 12 KJ/m^2d^1, Dias et al. (2018) reported decrease sucrose accumulations but an increase glucose and sorbitol levels in the olives. Additionally, Hamid et al. (2022) reported an increase in the sugar contents, including total soluble sugar, reducing sugar, and starch, both in roots as well as in leaves of white clover subjected to UV-B elicitation. Many of these studies supported the conclusions of this research and reported that plants store additional sugars as a defense strategy against various UV-B stresses.

Chlorophyll fluorescence signals measures photosystem II photochemical efficiency by considering the ratio of variable to maximal fluorescence (Fv/Fm). The maximum values of 0.80 to 0.85 represent an efficiency of 80 to 85% of the conversion of absorbed light into photochemistry. Any values less than this reveals less efficient photosystems and eventually photoinhibition of photosynthesis in plants. Under UV-B, the observed ratio in this experiment was lower than 0.80 which was previously recorded under UV-B and UV-C (Xu et al. 2022). It was concluded that the negative effect on Fv/Fm under UV-B is far adverse than UV-C irrespective of the irradiated wavelengths however, these findings are contradictory to the findings of (Shahzad et al. 2021) where under prolonged UV-B stress, rice plant regulated key metabolites to combat stress and maintained the Fv/Fm as well as net photosynthetic rate. In addition, it is a proven fact that the photosystem II is better adaptive and resilient to drought conditions than photosystem I (Khalaji et al. 2016) but when drought in combination with UV-B was subjected to Kale for 3 to 4 days, the quantum efficiency of photosystem II suffered a great decrease compared to drought alone and control (Yoon et al. 2020). In almonds, Fv/Fm value as low as 0.75 was recorded under UV-B stress which led to a substantial loss in total chlorophyll and net photosynthesis (Ranjbarfordoei et al. 2011). The maximum (Fv/Fm) and photochemical energy conversion into photosystem II were both reduced after administering UV-B to the *Arabidopsis thaliana* mutant *rsr4-1* and the *C24* wild type, although the mutant experienced more losses than the wild type (Czégény et al. 2019). Sweet basils showed serious symptoms of UV damage, an inhibition in the photosynthetic machinery and significant drop in the maximal quantum efficiency of photosystem II after being exposed to a UV-B (Mosadegh et al. 2021). The results of previous studies as well as this present research clearly depicted that UV-B as a stress is responsible for the decrease in quantum yield, quantum efficiency of photosystem II and overall performance index of plants.

5. Conclusions

Results of the present study show that plants contracted UV-B as a stress and behaved accordingly by releasing phytochemicals in response to the varying UV-B doses applied. Reduction in the *chl a* and *b* as well as carotenoids was observed in multiple UV-B doses. UV-B induced an enhanced phenolic index, anthocyanins and glucosinolates in almost all the UV-B treatments which clearly depicted the role of phytochemicals in plant defense mechanism against the stress. Moreover, a sharp increase for the nitrate contents has been recorded. Increase in the nitrates as well as decrease in the overall chlorophyll and photosynthetic pigments illustrated a disturbance in the nitrogen metabolism under UV-B applications. However, a buildup of sucrose, reducing sugars and total sugar was recorded which unfolds plant strategy of enhancing immune response against the UV-B. TBARS analysis revealed that the UV-B dose of 21.6 KJ/m^2 prompted less lipid peroxidation in the wild rocket while yielding an enhanced secondary metabolite compared to the other UV-B doses in which severe damage to the membrane has been noticed. Non-destructive analysis also demonstrated the role of UV-B as a stress, responsible for increased flavanols and anthocyanins but a decrease in chlorophyll estimation. Overall performance of the UV-B induced plant suppressed and a decline in the maximum quantum efficiency of photosystem II was recorded. So, for future studies, we recommend UV-B dose of 21.6 KJ/m^2 for enhancing and investigating the secondary metabolites in wild rocket. Moreover, it should be kept in mind that UV-B intensity, exposure time as well as the proximity of UV-B source should be carefully adjusted in order to obtain the maximum of secondary metabolite production from UV-B irradiation.

CRediT authorship contribution statement

Awais Ali: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Giulia Franzoni:** Data curation, Writing – review & editing. **Alice Petrini:** Data curation, Writing – review & editing. **Piero Santoro:** Resources. **Jacopo Mori:** Resources. **Antonio Ferrante:** Conceptualization, Methodology. **Giacomo Cocetta:** Conceptualization, Methodology, Data curation, Writing – review & editing.

Declaration of Competing Interest

The author Piero Santoro is employed by the company MEG Science. The author Jacopo Mori is employed by the company ALMECO S.p.a. All other authors declare no competing interests.

Data availability

Data will be made available on request.

Acknowledgements

Authors would like to acknowledge the efforts of Noramon Tantashutikun for her help in making graphical abstract and flow sheet diagram of UV-B effects in plants.

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