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ORIGINAL PAPER

Effect of UV-B elicitation on spearmint's (*Mentha spicata* L.) morpho-physiological traits and secondary metabolites production

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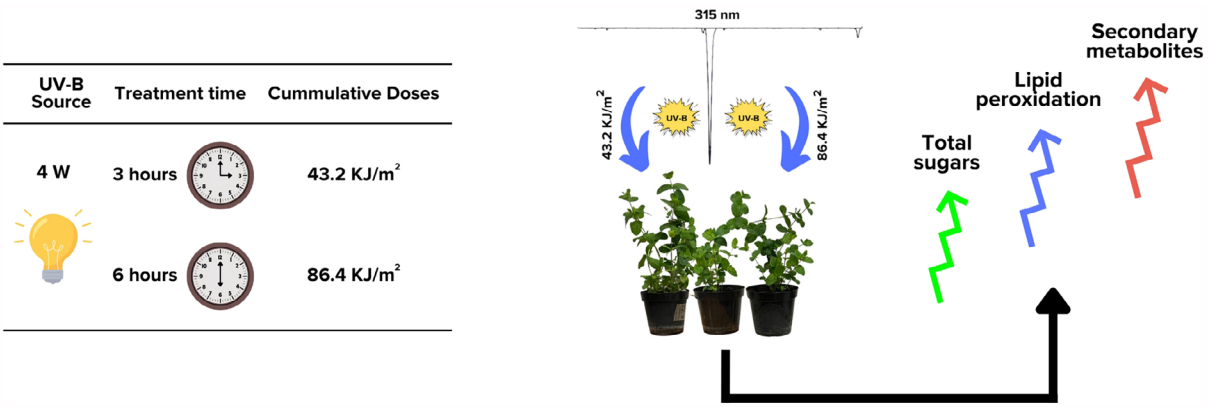
Abstract

Additional artificial ultraviolet-B irradiation (UV-B) is a substitute for the natural UV-B radiation, which is believed to modulate the antioxidants production in plants against the abiotic stress. The present research was formulated by using an indoor UV-B chamber, capable of irradiating UV-B at single wavelength of 315 *nm* on spearmint. In vitro destructive analysis revealed the reduction in accumulation of chlorophyll *a* and *b* as well as carotenoids. However, an elevated levels of phenolic index and total anthocyanins were recorded for both 43.2 KJ/m^2 and 86.4 KJ/m^2 UV-B doses. TBARS assay was carried out to record the lipid peroxidation in the spearmint and likewise, an enhanced secondary metabolites production, an increased lipid peroxidation was seen with the successive increased in the UV–B doses. A remarkable accumulation in the total sugars, reducing sugars and sucrose were also noticed. In vivo analysis revealed a decrease in chlorophyll estimated values as well as an increase of flavanols. Overall leaf fluorescence performance index was improved under both UV-B doses as well as the maximum quantum efficiency of photosystem II. Decreases in the total yield, plant height and length of internode was observed for both UV-B doses however, an enhanced number of stems was recorded for the 43.2 KJ/m^2 UV-B dose. The results of present study not only provided a clear indication of the potential of UV-B in modifying the phytochemicals accumulation in plants but also opened a room for investigating various indoor UV-B doses for future studies of plants and their interaction towards UV-B.

Graphical Abstract



Keywords Elicitors · Lipid peroxidation · Total sugars · Carotenoids · Antioxidants · Chlorophyll fluorescence

Introduction

One of the most significant environmental light factors influencing plant growth and development is ultraviolet B (UV-B) radiation. Both natural sunlight and artificial sources, including fluorescent lamps, expose plants to UV-B

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radiations in response to which plants may suffer harm due to genetic mutations, DNA damage or cell death (Bhusal et al. 2021). Additionally, UV-B can change genes activation, expression and subsequent protein translation. Numerous mechanisms, such as the biosynthesis of phytochemicals, antioxidants, and secondary metabolites, are involved in directing UV-B effects on plants. Tocopherols, phenolic compounds, carotenoids, anthocyanins and flavonoids are some of the most significant photo protectants which absorb UV-B radiation and turn it into heat, eventually plant cells are not harmed. Furthermore, these substances also scavenge radicals created by UV-B exposure and can provide plants with several advantages, such as increased resistance to pests and diseases or delayed leaf senescence (Jiao et al. 2016). Effects of UV-B on plants are well known. More phytochemicals and antioxidants are produced by plants that are exposed to UV-B radiation (Khaleghi et al. 2019). These secondary metabolites aid in shielding the plant from UV-B radiation's harmful effects.

UV-B is a highly dynamic ecological component in the natural world, and depending on the time of day, season, and weather, its levels can change significantly. Plants respond to UV-B radiations in two suggested signaling pathways. Cytosolic UVR8 photoreceptor control UV-B-specific signaling pathway that results in UV-B protection and morphological changes in plants. When UVR8 participates in signaling, it binds to the multifunctional E3 ubiquitin ligase constitutively photomorphogenic 1 (COP1), monomerizes, and translocates into the nucleus where it inhibits the degradation of the photomorphogenic transcription factor elongated hypocotyl 5 (HY5). HY5 and its homolog (HYH) successively control genes producing the phenylpropanoid pathway enzymes phenylalanine ammonia lyase (EC. 4.3.1.24), chalcone synthase (EC. 2.3.1.74) and flavanol synthase (EC 1.14.20.6), among other key elements involved in UV acclimation response and UV protection (Christie et al. 2012; Heijde and Ulm 2012; Schreiner et al. 2016a, b). Unrelated to UV–B signaling, one such mechanism hypothesizes that molecular disintegration and/or an accumulation of signaling molecules like ROS and molecules or phytohormones involved in injury or defense, like jasmonic acid, salicylic acid, nitric oxide, and ethylene, may be the root causes of UV–B induced oxidative stress responses (Cisneros-Zevallos et al. 2014; Vranová et al. 2002). Consequently, signaling pathways linked to damage and defense, activate stress-associated genes which then become overexpressed e.g., PR-1, PR-2, PR-5 and the defense gene PDF1.2 as represented in Fig. 1.

Spearmint (*Mentha spicata* L.) which belongs to genus Mentha in Lamiaceae family, was originated by a cross between *M. longifolia* and *M. rotundifolia* (Lawrence 2006). The world's temperate and sub-temperate zones make up the majority of its distribution. Modern cuisines like mojitos and Asian fusion dishes like Thai and Vietnamese both use fresh spearmint as a primary ingredient. Additionally, the biological actions of carvacrol, menthol, carvone, methyl acetate, limonene, and menthone found in spearmint's essential oil compositions are all known, which may lead to a market for it as a functional food ingredient (Sommano et al. 2022; Wu et al. 2019). Since spearmint is a hybrid, it seldom reproduces from seeds and instead only grows from its vegetative components and by micropropagation. In the current study, secondary metabolites of spearmint were investigated after growing it indoor and treated with UV-B of wavelength 315 *nm* and two UV-B doses of 43.2 KJ/m^2 and 86.4 KJ/m^2 for varying time span respectively. The aim of the approach was to evaluate UV-B stress and alterations in different morphological as well as physiological attributes. In vivo non-destructive estimation of chlorophyll fluorescence (Fv/Fm) and performance index (PI), various destructive analyses such as chlorophyl *a* and *b*, carotenoids, phenolic index, anthocyanins as well as TBARS were performed. Nitrate concentration, total sugars, reducing sugar and sucrose contents were also determined. Morphological traits such as yield, plant height, length of internode and number of stems were evaluated.

Materials and methods

Plant material

Spearmint (*Mentha spicata* L.) plant, was used as planting material and identical rhizomes, were sown in pots filled with a peat-based substrate. Cultivation took place in an experimental greenhouse under monitored growing conditions (24 ± 2 °C) with the addition of supplemental LED lights (photoperiod of 16 h) 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}) and 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.

UV-B exposure to spearmint

In the trial, two different treatments, one intense and one less intense, were tested, resulting in cumulative energies of 43.2 KJ/m^2 and 86.4 KJ/m^2, respectively.

For UV-B treatments, an indoor UV chamber equipped with an aluminium reflector realized with vega® UV, a PVD surface specifically developed to optimize the reflectance in the UV bandwidth, a wavelength of 315 *nm* as shown in Fig. 3, was used. All treatments have been performed

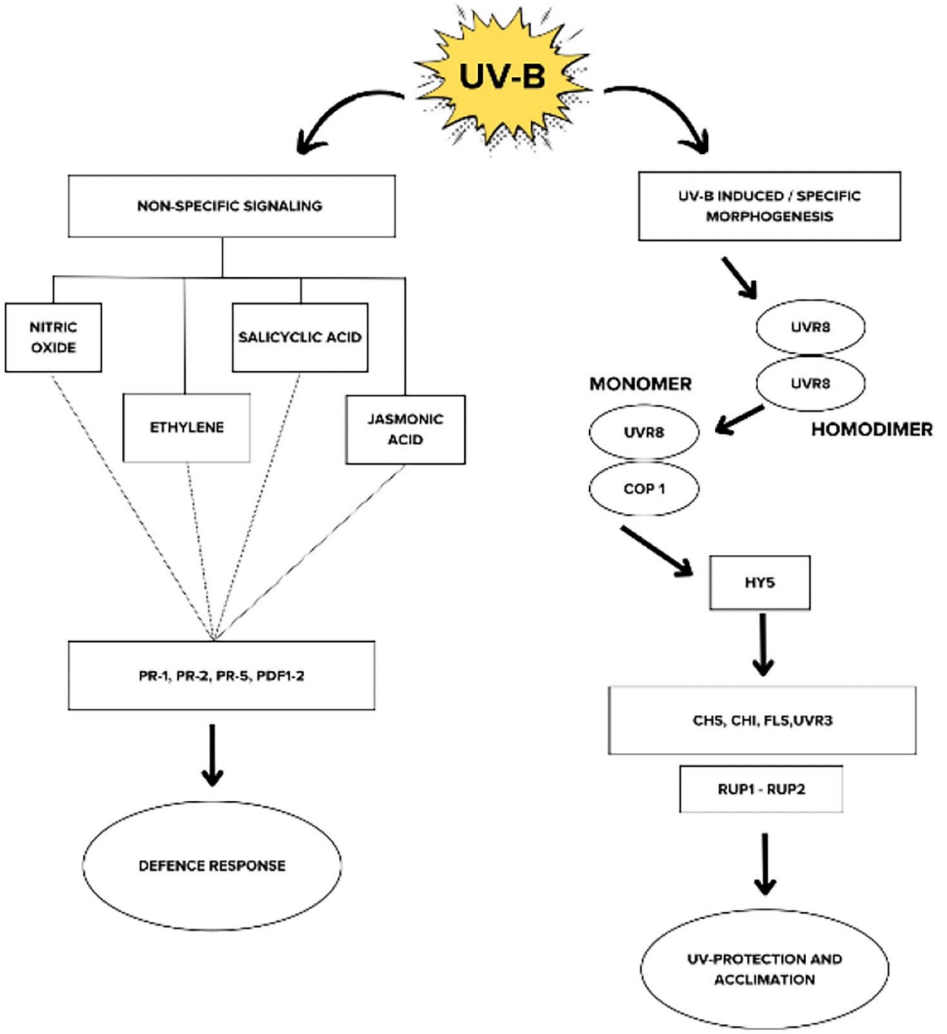

Fig. 1 Schematic responses of UV-B specific and non-specific signaling in plants

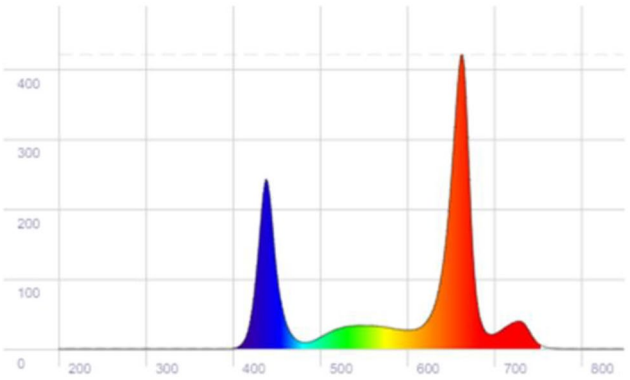

Fig. 2 Spectral quantum distribution used in glasshouse for spearmint (R : 77.1% ; G + Y :17.9% ; B : 5%)

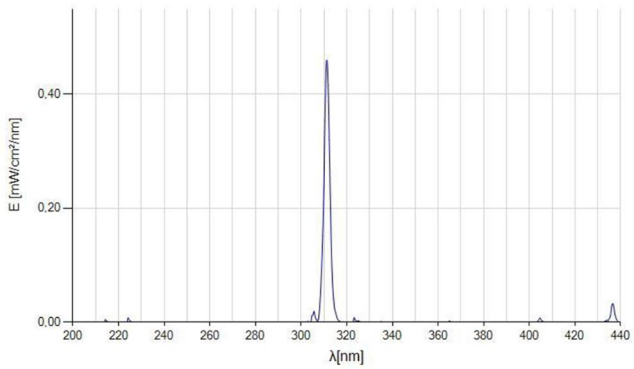

Fig. 3 Spectrum of the UV-B lamps. A single wavelength of 315 *nm* was subjected on the spearmint plants under trials

overnight with an automatic on and off mode as shown in Table 1.

When plants were sufficiently developed (10–12 cm height), a day per week of UV-B treatment (for 3 consecutive weeks) from a 4 W UV-B source was administered three times for the time exposure of 6 h and 3 h per day for cumulative 86.4 KJ/m^2 and 43.2 KJ/m^2 UV-B doses respectively. Non-destructive in vivo tests were performed by using 100

Table 1 Time scheme for an automatic UV-B treatments of both UV-B 43.2 KJ/m^2 and 86.4 KJ/m^2 doses to spearmint. For 43.2 KJ/m^2, treatment started at 20:00 pm and lasted for 30 min after which there was an off-phase for next 1 h and 30 min. Treatment started again at 22:00 pm for another 30 min UV-B exposure followed by another 1 h and 30 min off-phase and so on until 06:30 am for total 3 h of UV-B treatment. For 86.4 KJ/m^2, starting time was 20:00 pm and UV-B exposure of 1 h was applied to spearmint after which there is an off-phase of 1 h. UV-B treatment started again at 22:00 pm for 1 h until 23:00 followed by a 1 h off-phase and so on until 07:00 am for total of 6 h of UV-B treatment

UV-B 43.2 KJ/m^2			UV-B 86.4 KJ/m^2		
ON	OFF	Minutes	ON	OFF	Minutes
20:00	20:30	30	20:00	21:00	60
22:00	22:30	30	22:00	23:00	60
00:00	00:30	30	00:00	01:00	60
02:00	02:30	30	02:00	03:00	60
04:00	04:30	30	04:00	05:00	60
06:00	06:30	30	06:00	07:00	60
		Total: 3 hours			**Total: 6 hours**

MPM- Multi-pigment-meter and portable fluorimeter, the day after each UV-B treatment to determine the physiological conditions of plants. Only measurements obtained right before harvesting, nevertheless, will be displayed for in vivo results and discussion.

Total chlorophylls and total carotenoids

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

Phenolic index and total anthocyanins

For the extraction of the phenolic compounds, around 50 mg of matured 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 et al. 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 an extinction coefficient εM of 29,600 (Ferrante et al. 2004).

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 Dubnoff bath (PID) 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 $\varepsilon M = 155\ mM^{-1}\ cm^{-1}$.

Nitrate concentration

The nitrate concentration 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 1.5 N (NaOH) sodium hydroxide. 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).

Total sugars, reducing sugars and sucrose concentration

The extracts used were the same previously prepared for the nitrate determination. 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, 4.1 mM thiourea 1.5 M 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). Thereaction mixture was heated at 100 °C for 5 min, 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. In the next step, 0.5 mL of extract was placed on top of 2.5 mL of anthrone reagent and kept in ice for 5 min. the mix was vortexed vigorously and 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).

Total yield and morphological evaluations

The spearmint plants were cut from the base using scissor and weighed using measuring balance to investigate the fresh weight of the produce, by using a digital scale (Mettler PM480 Delta Range). Plant height was measured in centimetres using a measuring scale from the tip of the spearmint plant to the bottom where it was attached to the peat-based substrate. The total number of stems were counted manually by observing individual plants. Moreover, the length of internodes was measured in centimetres using measuring scale. The distance of one node to another was recorded by selecting the bottom nodes of each plant under UV-B treatments and control.

Non-destructive in vivo estimation of leaf pigments

After each UV-B application, as well as at harvest, Nitrogen-Flavonol Index, chlorophyll, flavonols and anthocyanin contents were measured in vivo using MPM-100 Multipigment meter (ADC BioScientific Ltd, UK) (Cerovic et al. 2008). Fully expanded leaves (the third leaves from the top of the plants were chosen) especially the adaxial surfaces were selected for in vivo determinations, to measure the above-mentioned parameters. However, while explaining the UV-B effect on spearmint only two parameters such as chlorophyll and flavonols were considered.

Non-destructive in vivo estimation of chlorophyll *a* fluorescence

This analysis has been performed 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) spearmint leaves (the third leaves from the top of the plants were chosen) using a portable fluorimeter (Handy PEA; Hanstech, Kings Lynn, UK). The parameters measured were the maximum quantum efficiency of photosystem II (Fv/Fm) and leaf fluorescence performance index (PI) which is derived from JIP test and it provided 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.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) followed by Tuckey's post-test with multiple comparisons test. Analyses were performed using GraphPad

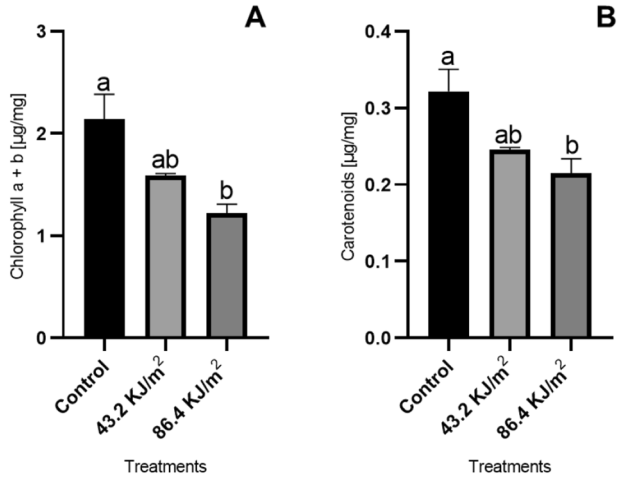

Fig. 4 (**A**) Chlorophyll *a* and *b*, (**B**) Carotenoids contents of spearmint treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses and control. Values are mean (n = 3 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p < 0.05$)

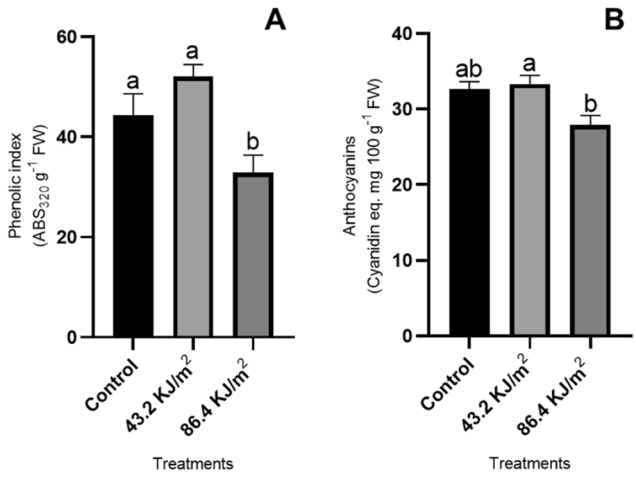

Fig. 5 (**A**) Phenolic Index (**B**) Anthocyanins contents of spearmint treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses. Values are mean (n = 3 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p < 0.05$)

Prism version 6 for Windows (GraphPad Software; La Jolla, California, USA, www.graphpad.com).

Results

Physiological and quality evaluation of spearmint for 43.2 KJ/m^2 and 86.4 KJ/m^2 UV-B doses

Referring to the Fig. 4, a significant decrease in the chlorophyll concentrations of UV-B treatment 86.4 KJ/m^2 was noticed (1.219 µg/mg) compared to the (2.140 µg/mg) of control while the 43.2 KJ/m^2 dose yielded a median non-significant chlorophyll concentration (1.587 µg/mg) compared to the control. Moreover, a similar trend has been observed for carotenoid's concentration in which 43.2 KJ/m^2 UV-B dose exceeded in the accumulation of carotenoid pigment (0.2458 µg/mg) against the (0.2151 µg/mg) of 86.4 KJ/m^2 UV-B dose. However, there were no significant differences observed between the two UV-B treatments in terms of carotenoids accumulation. An increased accumulation (0.3219 µg/mg) of carotenoids however was recorded in the control compared to both UV-B doses. Although, this increased carotenoid value was significant to UV-B treatment of 86.4 KJ/m^2, a non-significant difference has been recorded between control and 43.2 KJ/m^2 UV-B dose respectively.

Significant differences have been recorded between the two UV-B doses in terms of phenolic index in which UV-B 43.2 KJ/m^2 recorded the highest phenolic index accumulation among all the treatments. However, it was recorded lowest in the 86.4 KJ/m^2 UV-B dose and was significant compared to the control. Being the highest accumulated phenolic index of (52.05 ABS_{320} g^{-1} FW) in 43.2 KJ/m^2 UV-B dose, it was recorded (44.27 ABS_{320} g^{-1} FW) in control and (32.86 ABS_{320} g^{-1} FW) for 86.4 KJ/m^2 UV-B respectively. Likewise phenolic index, an increased significant anthocyanin (33.31 Cyanidin 3 -glucoside eq. mg 100 g-1 FW) was observed in 43.2 KJ/m^2 UV-B dose compared to the (27.93 Cyanidin 3-glucoside eq. mg 100 g^{-1} FW) of 86.4 KJ/m^2 UV-B treatment. Non-significant differences, however, were noticed between the control (32.65 Cyanidin 3-glucoside eq. mg 100 g^{-1} FW) and the two subjected UV-B treatments in the spearmint as shown in Fig. 5.

A reduction in the nitrate content was observed in the UV-B treated spearmint compared to the control as seen in Fig. 6. The UV-B 43.2 KJ/m^2 exhibited the significant lowest nitrate concentration (167 mg/Kg) compared to the (192.7 mg/Kg) of another UV-B dose of 86.4 KJ/m^2, making it the most prominent and advantageous of all treatments. Control however, recorded the highest nitrate production (210.8 mg/Kg) among all the treatments and was non-significant to the 86.4 KJ/m^2 UV-B dose. TBARS assay was performed to contemplate the damage cause by the UV-B to the membranes. An increase in the malonaldehyde formation was observed with the increase in the UV-B dose however, no significant differences were recorded among all the treatments. The control demonstrated the lowest value (213.7 nmol MDA g^{-1} FW) of lipid peroxidation against the (335.9 nmol MDA g^{-1} FW) and (343.8 nmol MDA g^{-1} FW) of 43.2 KJ/m^2 and 86.4 KJ/m^2 UV-B doses respectively.

An increase was seen in the total sugar contents in both UV-B doses of 86.4 KJ/m^2 and 43.2 KJ/m^2 compared to the control. A recorded total sugar value of (213.7 mg Glu

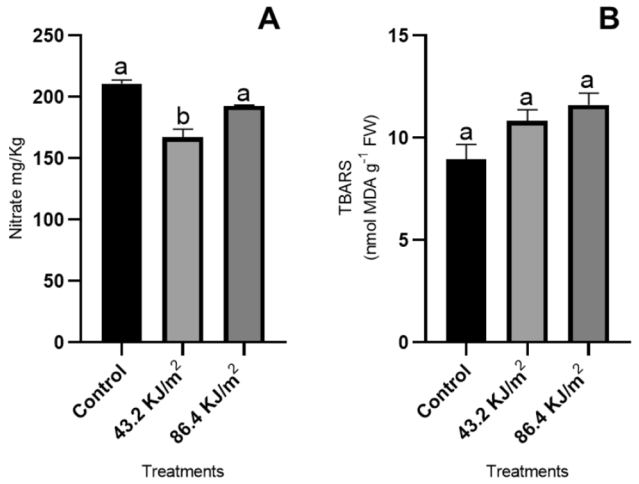

Fig. 6 (**A**): Nitrate (**B**) TBARS assay of spearmint treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses. Values are mean (n = 3 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison test ($p < 0.05$)

eq g^{-1} FW) in control was significantly lower to the ones recorded for UV-B treated spearmint such as (335.9 mg Glu eq g-1 FW) and (343.8 mg Glu eq g-1 FW) for 43.2 KJ/m^2 and 86.4 KJ/m^2 respectively. However, no significant differences found in the total sugar contents between the two UV-B treatments as per Fig. 6. Like total sugars, a similar trend has been observed for the reducing sugars in which an enhanced reducing sugar contents for both UV-B treatments were recorded. A strong significant difference has been observed between UV-B treatments and control, with control being the lowest 56.21 mg/Kg, 86.4 KJ/m^2 being the next with 81.42 mg/Kg and the recorded high reducing sugar value of 87.78 mg/Kg for the 43.2 KJ/m^2. An enhanced sucrose accumulation has been investigated in UV-B treated spearmint compared to the control. A non-significant difference was recorded between the control (2.834 mg/g) and the UV-B dose of 86.4 KJ/m^2 (3.230 mg/g). Furthermore,

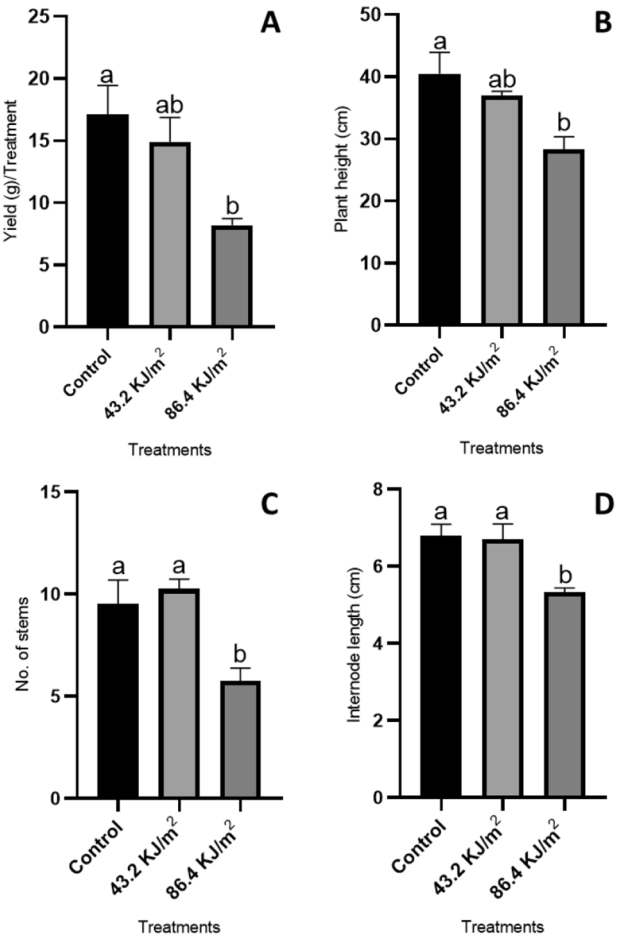

Fig. 8 (**A**) Total yield, (**B**) Plant height, (**C**) Number of stems, (**D**) Length of internode of spearmint treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses. Values are mean (n = 3 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison ($p < 0.05$)

the UV-B dose of 43.2 KJ/m^2 turned out the dose with significantly highest accumulated sucrose among all the

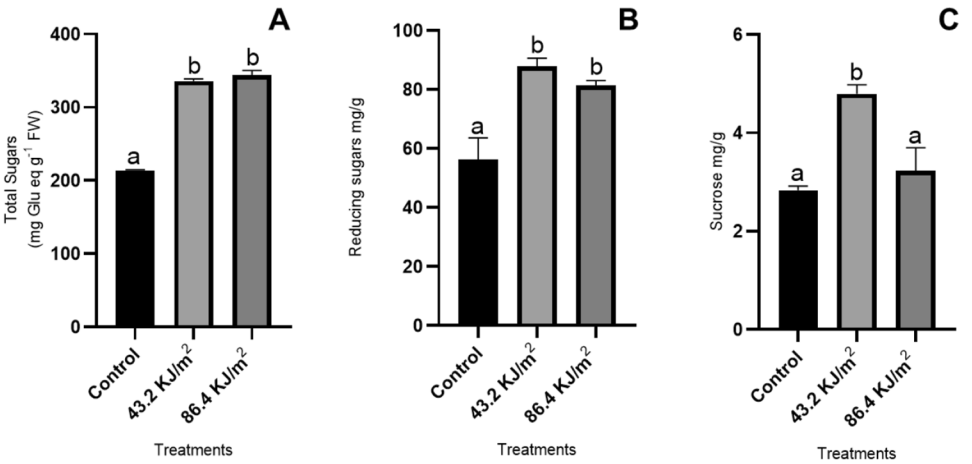

Fig. 7 (**A**) Total sugars, (**B**) Reducing sugars, (**C**) Sucrose contents of spearmint treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses. Values are mean (n = 3 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison ($p < 0.05$)

treatments with an observed sucrose level at (4.791 mg/g) as represented in Fig. 7.

Total yield and morphological evaluations of spearmint for the UV-B Doses of 43.2 KJ/m ^{2} and 86.4 KJ/m^2

According to Fig. 8, reduction in the spearmint yield has been observed with the successive increase in the doses of UV-B irradiations. Control demonstrated a significant highest total yield of spearmint (17.10 g/treatment) against the lowest yield (8.195 g/treatment) of 86.4 KJ/ m^2 UV-B dose. The UV-B dose of 43.2 KJ/m^2 however, was non-significant to the above-mentioned treatments and yielded (14.91 g/treatment), a yield intermediate to the control and 86.4 KJ/m^2 UV-B dose. UV-B irradiation inversely affected the plant height with a pattern like the one observed in the yield of the spearmint. The highest significant plant length was measured in control (40.50 cm) against the lowest (28.33 cm) in 86.4 KJ/ m^2 UV-B however, a non-significant reduction in plant height (37 cm) was observed for 43.2 KJ/m^2 UV-B dose. Furthermore, an increased but non-significant number of stems were observed in 43.2 KJ/m^2 UV-B dose compared to the control. While the 86.4 KJ/m^2 UV-B dose recorded the significant lowest number of stems against the control. Length of internode was also considered while treating the spearmint with UV-B and it has been found that with the increase in the UV-B dose, there was a decrease in the length of internode. The UV-B dose 86.4 KJ/m^2, being the intense UV-B treatment, resulted in a significant reduced length of internode compared to the control. However, the 43.2 KJ/m^2 UV-B treated plants demonstrated a moderate value for length of internode among the tested treatments.

Non-destructive estimation of chlorophyll and flavanols by using MPM 100 multi-pigment-meter

In vivo non-destructive estimation of chlorophyll and flavanols have been carried out using MPM-100 Multi-pigment-Meter. A decrease in chlorophyll has been estimated for both UV-B treatments such as 86.4 KJ/m^2 and 43.2 KJ/m^2 compared to the control and was significantly different between 86.4 KJ/m^2 UV-B dose and the other two treatments as represented in Fig. 9. Flavanols were enhanced in response to both UV-B treatments however, no significant differences have been recorded among the treatments.

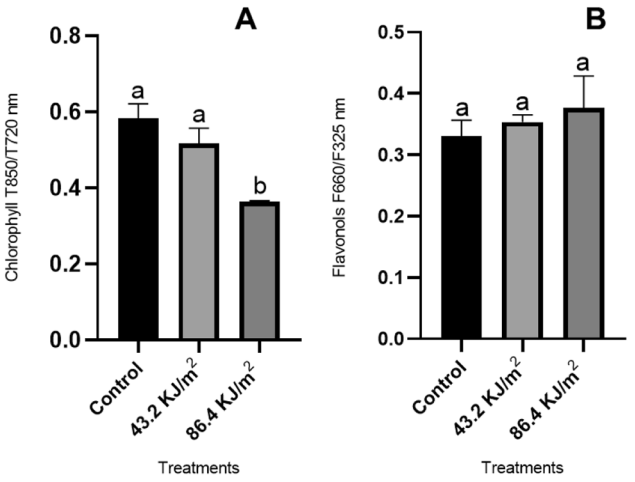

Fig. 9 In vivo non-destructive estimation (**A**) chlorophyll and (**B**) flavanols of spearmint grown as control and treated with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses using MPM-100. Values are mean (n = 4 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison ($p < 0.05$)

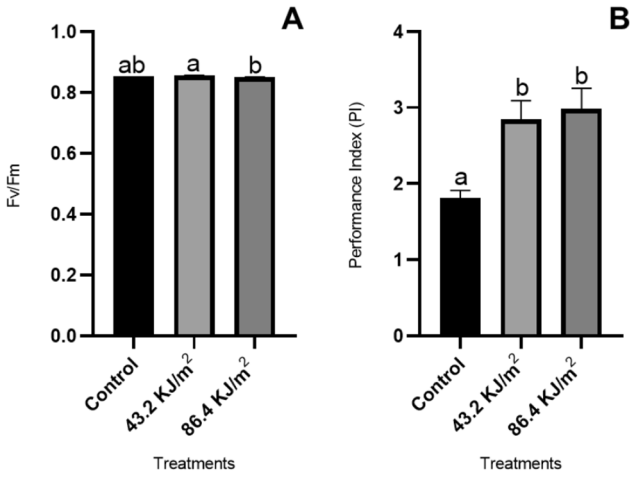

Fig. 10 Non-destructive measurements using fluorimeter (**A**) Maximum quantum efficiency of photosystem II (*Fv/Fm*) (**B**) Leaf fluorescence performance index of spearmint. The figure represents control and UV-B treated spearmint with 43.2 KJ/m^2 and 86.4 KJ/ m^2 UV-B doses. Values are mean (n = 4 ± S.E). Different letters indicate significant differences among treatments followed by Tukey multiple comparison ($p < 0.05$)

Non-destructive estimation of maximum quantum efficiency of photosystem II (Fv/Fm) and performance index (PI) by using fluorimeter

By using Handy PEA + fluorimeter, an in vivo estimation of maximum quantum efficiency of photosystem II (Fv/Fm ratio) as well as the overall leaf fluorescence performance index has been evaluated. The results are represented in the Fig. 10 below. Under UV-B stress it has been noted that the photochemical efficiency of photosystem II increased

significantly in 43.2 KJ/m^2 UV-B dose compared to the 86.4 KJ/ m^2. However, all the treatments recorded the values above 0.85, representing the maximum possible photosynthesis efficiency of spearmint under tested treatments. Interestingly, non-significant increase was observed among the two UV-B treatments compared to the control, which showed a significant reduced values compared to the UV-B treated spearmint.

Discussion

Over the years, various approaches have been in used to modify the genetic makeup and modulate the plant tolerance to several environmental stresses. Mutagenesis such as chemical and irradiation, gene editing, cloning and marker assisted selections provided an ample attribute to crops (Awais et al. 2019). Ultraviolet radiation technology may be a simple and effective way to stimulate the production of healthy ingredients in fruits and vegetables. Despite functioning as an environmental stressor, UV-B caused a variety of alterations in different species, and its outstanding regulatory role in plant growth and secondary metabolism. Therefore, where studies have shown that UV-B has a deleterious impact on plants, there is also evidence to support its importance as a tool for plant enhancement initiatives.

In vitro and in vivo quality evaluation of spearmint (*Mentha spicata* L.) for accumulation of secondary metabolites

In vivo as well as in vitro evaluation in this study suggested a drastic decrease in the chlorophyll when subjected to the UV-B doses of 43.2 KJ/m^2 and 86.4 KJ/m^2. To increase the possibility of photosynthesis, chlorophyll helps plants collect extra light, convert it into photosynthetic electron transport, and then use the end product as an energy. The loss of chlorophyll in this study with UV-B dosages of 43.2 KJ/m^2 and 86.4 KJ/m^2 may be explained by the destruction of UV-B damage to the chloroplast followed by the degeneration of photosystem II. When *Arabidopsis thaliana* was exposed to UV-B, senescence-related genes were activated, causing a decrease in total chlorophyll and designating UV-B as an abiotic stress (Sztatelman et al. 2015). A similar detrimental effect of chlorophyll *a* and *b* was recorded in corn (*Zea mays)*, rice (*Oryza sativa)*, almond *(Prunus dulcis)* and silvergreen bryum moss *(Bryum argenteum)*, when subjected to varying UV-B doses, the results which are in complete favor of present findings (Jovanić et al. 2022; Lidon and Ramalho 2011). Furthermore, when exposed to UV-B, common duckweed (*Lemna minor*) showed a 20% drop in chl *b*, whereas chl *a* did not show any discernible effects of that study (Xie et al. 2022). Contrary to the present findings, an increase in total chlorophyll has been observed in the leaves of *Rosa hybrida* and *Fuchsia hybrida* (Helsper et al. 2003). In contrast, Paccini et al. (2020) study of two Italian olive varieties revealed no changes in the chl *b* in Olivastra Seggianese variety, but a decrease in pigment accumulation was recorded in Giaraffa olive variety. Despite the fact that UV-B is to hold responsible for the reduction in the total chlorophyll content of the plants, these variations in the results suggest that this effect can be explained as dose or specie dependent, taking into account the decrease, increase or lack of an impact of chlorophyll augmentation against UV-B stress.

When spearmint was exposed to artificial UV-B stress, the production of carotenoids declined, with a pronounced decline seen at higher UV-B doses. Carotenes and xanthophylls are the two main subgroups of carotenoids, which are natural compounds found in photosynthesizing species (Sun et al. 2018). While xanthophylls aid plants in protecting the chlorophyll from photooxidation, carotenes are important in eliminating reactive oxygen species and in harnessing light (Shen et al. 2018). The specific regulatory roles of the genes involved in carotenoids metabolism are yet unknown under UV-B irradiation, although the expression of many critical pathway's genes, including *PSY*, *LCY-E*, *LCY-b*, *CHY-b*, and *VDE*, is crucial for the accumulation of carotenoids in plants (Toledo-Ortiz et al. 2010; Welsch et al. 2007). Being protective against UV-B and ROS in nature, the decrease in production of carotenoids instead of increase, is mainly because of the specific and non-specific signaling pathways of UV-B transduction. It has been found previously in canola seedlings that under low UV-B doses such as 5 KJ/m^2d^1 and 10 KJ/m^2d^1, there is always an increase in the carotenoid's accumulation, hence implying the role of UV-B specific pathways (Qaderi et al.2010). Additionally, and particularly under strong UV-B doses as those in our experiment, carotenoids biosynthesis might be indirectly impacted by non-specific signaling cascades linked to significant DNA damage and excessive ROS generation (Jansen et al. 2008). Reduction in carotenoids has also been reported by (Nazari and Zarinkamar 2020) while observing the response of UV-B irradiation on *Mentha aqautica*. It's important to note that a low artificial preharvest treatment of 0.54 kJ/m^2d^1 UV-B led to an accumulation of 68% lycopene and 41% ß-carotene, respectively, following 22 h of adaptation of tomato fruits of the cultivar Liberto (Perez et al., 2008). However, when UV-B exposure was combined with other environmental factors like water stress, the results were computed in the opposite and unfavorable ways which led to the decrease in carotenoids (Qaderi et al. 2010). Hence in order to precisely understand the role of UV-B in

the accumulation of carotenoids, UV-B exposure time, number of days of exposure and intensity must be adjusted.

Most research to date has been on figuring out how UV-B exposure affects levels of phenolic compounds in plants including flavones, flavanols, isoflavonoids, anthocyanins, and phenolic acids. These phenolic compounds tend to be found mostly in the outer surfaces of plants where they serve as a protective barrier against extended exposure to high doses of solar radiation, particularly its UV-B component (Soobrattee et al. 2005; Dixon 2001). In vivo analysis of flavanols indicated an increased accumulation in both UV-B doses compared to the control whereas in vitro analysis revealed that UV-B dose of 43.2 KJ/m^2 turned out to be an effective dose for efficient accumulation of both phenolic index as well as anthocyanins. The expression of phenolic and flavonoid genes, such as those for chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavonol synthase (*FLS*), dihydroflavonol 4-reductase (*DFR*), and phenylalanine ammonia lyase, is regulated during the UV response by the UV-responsive transcription factors *HY5* and *PFG1/MYB12* (Gai et al. 2022; Stracke et al. 2010). The effect of UV-B on plant chemistry is also transient and time-dependent, according to Hectors et al. (2014). A similar study carried out on pigeon pea root cultures exposed to UV-B exposure for 2 to 4 h had shown greater levels of phenolic compounds, which then gradually decreased over the following period up to 24 h. It was summarized that the slow fall was likely caused by the metabolic flux competition between *CHS* and *STS* (Gai et al. 2022). Additionally, prior studies found that production of flavanoids and flavonoids glycosides is more sensitive to UV-B than that of phenolic acids, suggesting that not all phenolic compounds are equally formed under UV-B stress (Screiner et al. 2016).

Nitrate transporters (*NRT*) are involved in nitrate uptake, movement, and dispersion in plants (Kant 2018). Nitrate is used by plants along with sugars to make amino acids and proteins once growth and primary metabolism are complete, making it the preferred nitrogen source for plants during growth and development (Ncube et al. 2012). In the current research, it was found that the nitrate content of both UV-B treatments decreased following the UV-B exposure which in return is a better explanation of an increased total sugar accumulation in UV-B treated spearmint. The nitrate transporter (*NRT1.8*) has been recently revealed to be implicated in increased nitrate buildup in shoots by 14.49 times (Wang et al. 2022). UV-B, in general increases the ROS in plants which triggers the activity of nitrate reductase (*NR*), followed by the conversion of nitrates into nitrites which eventually yielded nitric oxide (*NO*) under NR activity. NO activates the action of *CHS* and *CHI* genes, the one involved in UV-B protection by enhancing flavonoids production (Gupta et al. 2011). Several 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) while studying the behavior of nitrate reductase (*NR*) activity towards UV-B. Moreover, an impaired nitrogen metabolism has been reported previously under the elevated levels of UV-B (Choudhary and Agarwal 2014) while an increased antioxidant activity was seen while subjecting a foreign N supply to plants under UV-B stress (Yao and Liu 2006).

TBARS (Thiobarbituric Acid Reactive Substances) assay which indicates the UV-B oxidative stress experienced an increase in both UV-B treated spearmint compared to the control. Moreover, an increase production of these substances has been observed in the intense UV-B irradiations which are in line with the findings of Rai et al. (2011), who noticed an increase of 25% of these substances in UV-B treated *Artemisia annua.* Similarly, in a study where wheat seedlings were exposed to UV-B radiation it was observed after 90 min of UV-B stress that TBARS increased by 76%; yet, at the same time, under UV-B + melatonin treatment, TBARS fell to 35%, highlighting the melatonin's stabilizing benefits (Tian and Lei 2007). Superoxide dismutase (SOD), an antioxidant enzyme essential for physiological defense mechanisms in plants against free radicals and reactive oxygen species under biotic and abiotic stresses, has been found to be compromised by UV-B exposures, which increases TBARS values in ascorbate-deficient vtc1 mutants of Arabidopsis thaliana (Gao and Zhang 2008). Another investigation on tomato roots and leaves found that following exposure to UV-B stress, lipid peroxidation in the leaves increased by 18%; however, no increase in lipid peroxidation in the roots has been recorded (Mannucci et al. 2020). Therefore, the severe UV-B dosages in our experiment may have compromised the spearmint's ability to defend itself against UV-B effects, which led to a continuous rise in TBARS after the increased irradiations.

When exposed to UV-B stress for both doses, spearmint responded by producing more total sugar, reducing sugars, and sucrose. Sugars play a function in the interaction of signaling molecules, including phytohormones, in addition to being a crucial component of the defense mechanism against biotic and abiotic stress and a regulator of growth and development (Smeekens et al. 2010; Ciereszko 2018). The irradiated spearmint, which had a compromised nitrogen metabolism and produced fewer nitrates instead in order to provide energy for the defense mechanism against intense UV-B irradiations. Similar research on tomato plants exposed to UV-B found that whereas starch levels

changed over time under UV-A and UV-B exposures, sugar levels increased in the tomato plants. According to studies on white clover, the sugar content has increased in both the roots and the leaves, including total soluble sugar, reducing sugar, and starch (Mariz-Ponte et al. 2021). Furthermore, the addition of nitrogen, phosphate, and potassium to UV-B radiation significantly boosted the amount of soluble sugar in peas (Singh et al. 2015). Contrary to our findings, records of decreased sucrose accumulations and increased glucose and sorbitol levels in olive cultivars have been found with a UV-B dose of 12 KJ/m^2d^1 (Dias et al. 2018; Piccini et al. 2021). Numerous of these studies concurred with the findings of the current study and claimed that plants store extra sugars as a defensive mechanism against distinct UV-B stressors.

The spearmint yield decreased in comparison to the control due to a number of changes, including decreased chlorophyll, increased sugars, and antioxidant activity for the defense mechanism, which may be related to the buildup of reactive oxygen species in plant cells. Additionally, against both UV-B stress, a definite decrease in plant height and length of internode between the leaves was observed. But there was a slight increase in biomass and herb output that was seen and also reported in the earlier studies (Bassman et al. 2002). Rai et al. (2011) also noted morphological changes, including a reduction in plant height, a reduction in leaf area, and stem elongation. Intriguingly, in addition to different morphological changes, Rai and Agarwal (2020) discovered that *Eclipta alba* did not experience any changes in number of stems and length of internode which is in contrast with our finding in which we found that 43.2 KJ/m^2 resulted in increased number of stems after UV-B exposure. However, UV-B irradiation, a decrease in internode length has been noted in cucumber (Qian et al., 2020). Additionally, (Barickman et al. 2021) showed a decrease in the height of basil plants, but no discernible variations were noted for node lengths.

The photochemical effectiveness of photosystem II has been assessed using the ratio of variable to maximal fluorescence (Fv/Fm). The conversion of absorbed light into photochemistry is taken into account, and values above 0.80 indicate an efficiency of more than 80%. Values below this indicates fewer effective photosystems, which may eventually cause photoinhibition of photosynthesis in plants (Torres et al. 2021). Interestingly. spearmint have shown the efficiency of above 85% in all the treatment with 43.2 KJ/m^2 being the most efficient among all the treatments. Interestingly all UV-B treated plants have shown a positive overall performance index which also depicted a positive response of spearment towards the treated UV-B doses. These results are consistent with those of Shahzad et al. (2021), who found that under prolonged UV-B exposure, rice plants controlled key metabolites to reduce stress and kept their Fv/Fm and net photosynthetic rate constant. Contrary to our findings, it has been found that under UV-B stress, Fv/Fm values in almonds as low as 0.75 were seen. This resulted in a significant loss of total chlorophyll and net photosynthesis (Ranjbarfordoei et al. 2011). Higher Fv/Fm values were interpreted in various Arabidopsis accessions as well cultivated under high UV-B dose represented larger protective capability and an increased UV-B tolerance (Biswas and Jansen 2012).

Conclusions

The current findings demonstrate that plants experienced UV-B as a stressor and responded accordingly by producing phytochemicals. In spearmint that had been exposed to UV-B radiation, a decrease in photosynthetic pigments, *chl a* and *chl b* as well as carotenoids was noticed. Almost all UV-B treatments induced higher phenolic index and anthocyanin levels, as shown during in vivo and in vitro analyses, demonstrating the significance of these phytochemicals in spearmint stress defense mechanisms. In addition, there has been a drop in the nitrate levels in UV-B treated spearmint, which highlighted an involvement of nitrogen metabolism in the mechanism and regulation of UV-B responses. The overall performance index was positive among the UV-B irradiated plants including the normal display of maximum quantum efficiency of photosystem II. Also, a buildup of sucrose, reducing sugars and total sugar was recorded, consistently with a better performance of the PS II (as shown from the analysis of fluorescence) which unfolds plant strategy of enhancing the defense responses against the UV-B stress. TBARS analysis revealed that higher the UV-B dose, the higher would be the lipid peroxidation in plant cells. Moreover, morphological results indicated that plant suffered a decrease in overall yield, plant height and in length of internode after the UV-B exposure. However, there was an increase in the number of stems recorded for 43.2 KJ/m^2 UV-B dose compared to the control. In vivo analysis of chlorophyll and flavanols estimations revealed that plant suffered a decline in the chlorophyll values against the UV-B but responded with an increase production of flavanols. Results of this study suggested that UV-B has the potential to alter the morphological as well as physiological profile of the spearmint yet there is a sufficient room to modify the UV-B exposure, intensities, and crop species to obtain the better outcomes.

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Authors contribution AA, GC, AF: structured the experimental concept. AA: conducted the experiments, analyzed the data and wrote the manuscript. GC: assisted in experiments, data analysis, reviewed and edited the manuscript. PS and JM: manufactured the UV-B chamber for the experiment.

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Declarations

Conflict of 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.

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