

UV-A and UV-B combined with PAR intensity change in plant growth, total phenol, flavonoid, antioxidative and essential oil composition of *Pelargonium graveolens*

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Abstract

Background

Different wavelengths of solar radiation incident on earth [herein: Photosynthetically Active Radiation)PAR(,Ultra Violet-A)UV-A(and Ultra Violet-B (UV-B)] and their spectral balance not only have an impact on plants' growth, morphology and physiology, but also have been shown to be important for the quality and quantity of secondary metabolites produced by plants.

Material and Methods

In this outdoor study we addressed the effects of PAR intensity and UV on the growth, yield, antioxidative, phenolic, flavonoids and essential oil production of *Pelargonium graveolens* L'Hér. The experiment was performed as split plots in a randomized complete block design with three replications. During the growth, two PAR intensities (ambient PAR and reduced PAR) and four UV treatments (ambient UV, enhanced UV-A, enhanced UV-B and enhanced UVA + B) were applied.

Results

High PAR intensity declined the length and width of leaf, the height of plant and fresh weight of aerial part, but increased dry weight of aerial part. Enhanced UV-B irradiation was associated with reduced plant height, leaf expansion and fresh and dry weight of aerial part. Interestingly, negative influence of enhanced UV-B radiation on morphology and growth of plant was largely alleviated by high PAR intensity. The amount of total phenols and flavonoids, antioxidant activity and essential oil production of *P. graveolens* strongly increased with both enhanced UV-B irradiation and high PAR. On the contrary, UV-A radiation did not significantly influence total phenols, flavonoids, antioxidant activity and essential oil. Moreover, the combination of high PAR intensity and enhanced UV-B led to further increases in total flavonoid content and the antioxidant capacity. Both high PAR intensity and enhanced UV-B increased the percentage of geraniol in essential oil, leading to a slight reduction of C/G ratio.

Conclusions

Considering that the lower C/G ratio is the most important indicator of the high economic value of rose geranium essential oil, reducing C/G ratio under enhanced UV-B radiation and/or high PAR is likely to be favorable. Overall, we conclude that PAR and UV-B irradiation, as two important environmental factors, have positive effects on the quality and quantity of rose geranium secondary metabolites.

1. Background

Since the human activities have changed the Earth's atmosphere and consequently depleted the ozone layer, the investigation on the biological effects of ultraviolet (UV) irradiation has become an interesting issue in recent decades. On the Earth's surface, UV irradiation consists of a minor component (8–9%) of the full solar irradiation (Cockell and Knowland, 1999; Del-Castillo-Alonso et al., 2015). The same relatively low proportion, as an important environmental factor, induce diverse adaptive changes during life cycle and could change the adaptive mechanisms of plants to known stressor factors (Kovács et al., 2014).

UV radiation traditionally is divided into three parts based on the wavelength: UV-A (320–400 nm), UV-B (280–320 nm), and UV-C (200–280 nm) radiations. In plant systems each band can induce significantly different biological effects (Maharaj, 2015). UV-C quickly cause high levels of damage to cellular components (Stapleton, 1992). UV-C radiation is effectively absorbed by ozone and ambient gasses, and is not present at ground level in sunlight. UV-B irradiation has pleiotropic influences on the plant growth, development, biochemical response, physiology and morphology (Frohnmeier and Staiger, 2003; Sharma and Guruprasad, 2012). It acts as an eco-physiological factor that is capable to change metabolic switches between primary and secondary metabolism (Ballare et al., 2011). Adverse effects of UV-B radiation on generation of reactive oxygen species (ROS) which damages cell membranes, proteins, DNA, retard photosynthesis process and consequently plant growth, have frequently been reported (Müller et al., 2015; Vidovic et al., 2015; Shayganfar et al., 2018). UV-A radiation comprises a much higher proportion of the solar UV spectrum (up to 95% near the equator) (Brune et al., 2003; Verdaguer et al., 2017). This part of solar UV passes almost unaltered through the atmosphere and is capable to penetrate to inner parts of the tissue. However, it is considered less harmful per photon than UV-B (Liakoura et al., 2003; Kataria et al., 2012). Unlike UV-B, seasonal variation and different hours of the day significantly have little effect on daily UV-A flux (Brune et al., 2003; Seckmeyer et al., 2008; Verdaguer et al., 2017). UV-A can cause oxidative damage and growth inhibition in higher plants (Krzek and Chalker-Scott, 2005; Mumivand et al., 2021d). On the other

hand, UV-A radiation is moreover reported to mitigate the damaging influences of UV-B (Flint and Caldwell, 1996; Kataria et al., 2014). The increased levels of pigments and UV-absorbing compounds such as flavonoids, carotenoids, and antioxidants have been previously reported to enhance growth in general by supplementation of UV-A and photosynthetically active radiation (PAR: 400–700 nm) (Shiozaki et al., 1999; Helsper et al., 2003; Kataria et al., 2014).

In higher plants, light not only is an energy source but also acts as a key signal that regulates growth, development and metabolism of plant (Raffo et al., 2020). On the other hand, the spectral balance between photosynthetically active radiation (PAR) intensity, UV-A, and UV-B has been demonstrated to be significant in specifying plant response to UV irradiation (Krizek and Chalker-Scott, 2005). Plants have evolved a number of defense systems to cope with UV-B stress factors in nature. As an avoidance strategy, exposure of plants to UV-B radiation can result in increasing biosynthesis of antioxidant compounds such as essential oils, phenolics, carotenoids, as well as other natural products (Matsuura et al., 2013; Escobar et al., 2017). PAR intensity has also remarkable effects on plant growth, development and secondary metabolite biosynthesis. Thus, plant counteraction against the adverse effects of high PAR intensity and UV-B irradiation may overlap, imposing cross tolerance to both parts of sunlight (Bolink et al., 2001; Behn et al., 2010; Majer and Hideg, 2012).

Currently, field experiments attenuation and enhancement are two of the most suitable approaches that have been used to investigate plant responses to UV and PAR. Field attenuation studies are the most applicable from a spectral perspective, as plants are exposed to balanced ratios of UV/PAR (and depending on the treatment, UV-A: UV-B: PAR), which is greatly modified in greenhouse and growth chamber studies. Hence, supplementary UV-A and UV-B studies in the field studies are the most authentic resource of knowledge on the influences of UV irradiation and generate realistic data on the biological effects of various levels of UV-B and/or UV-A irradiation playing roles in regulating the development and growth of individual plants, plant communities or whole ecosystems (Verdaguer et al., 2017). Moreover, these experiments can give realistic sensitivity assessments and acclimation of plants to ambient UV-B and UV-A levels.

Rose geranium (*Pelargonium graveolens* L'Hér.), a perennial aromatic plant belonging to the family of Geraniaceae, is mostly cultivated for its essential oil which is economically valuable (Amiri et al., 2015). Rose geranium oil, obtained from the green foliage and flowers of the plant, finds extensive application in cosmetics, food, perfumery, and pharmaceutical industries (Rao, 2002; Rezaei Nejad et al., 2020). The essential oil is traditionally used as antioxidant, antibacterial, antifungal, antidiabetic, antiallergic, antidiarrhoeic, antihepatotoxic anti-inflammatory, anti-spasmodic, diuretic, and tonic (Ćavar and Maksimović, 2012; Patel and Patra, 2015). In aromatherapy, it is used as remedy for menopausal problems, nervous tension and anxiety, and as a skincare agent (Rao, 2002). The chemical composition of rose geranium oil has been the subject of several studies. Citronellol, geraniol, linalool, citronellyl formate, *p*-menthone, formatum, citronellium were found to be the major components of the essential oil (Boukhatem et al., 2013; Amiri et al., 2015; Benelli et al., 2017; Rosato et al., 2018).

Plant growth and morphology as well as essential oil production and constancy is strongly affected by the combined effects of the genetic factors and the environmental conditions (Nooshkam et al., 2017). Indeed, even though the essential oil content and composition is primarily under genetic control, its production is also particularly dependent on environmental factors including light intensity, UV irradiation, irrigation, nutrition, day length, temperature and drying method (Bian et al., 2015; Mumivand et al., 2021a; Taheri-Garavand et al., 2021; Beiranvandi et al., 2022). Therefore, the influence of PAR intensity and UV irradiation on essential oil production of rose geranium could have useful implications on management of product quality and standardization in open field cultivation. Hence, the present study aimed to investigate growth and yield, essential oil production, total phenol and flavonoids as well as antioxidant activity of *P. graveolens* exposed to different PAR intensity and UV-A and UV-B radiation.

2. Materials And Methods

2.1. Plant materials and experimental site

The experiment was carried out from early June to late September in two consecutive years 2020 and 2021. Stock plants of *Pelargonium graveolens* were obtained from Medicinal Plants Garden of Lorestan University, Lorestan, Iran. Healthy cuttings were collected from the mother plant and placed in plastic pots with a substrate of sand and kept in the greenhouse. Plants were raised under conditions of natural PAR irradiances (up to 600 $\mu\text{mol photons/m}^2/\text{s}$) and exclusion of UV-A and UV-B for 30 days. After root formation, the cuttings were transplanted into 8 L pots containing a substrate of soil, sand, and cow manure (1:1:1) as substrate. Then, rose geranium plants were transferred to the outside of greenhouse at the beginning of June. Acclimatization of plants to the conditions of open air was done two weeks prior to the PAR and UV treatments. The outdoor setting was conducted in the research garden of Lorestan University of Khorramabad (longitude 48°26' E, latitude 33°44' N, altitude 1170 m). During the experiment, plants were irrigated twice a week and fertilized with humic acid (2.5 mL/L) twice.

2.2. UV and PAR treatments

The experiment was done as split plots in a randomized complete block design with three replications. Two PAR intensities (ambient PAR and reduced PAR) (as main plots) were applied, as well as four UV treatments (solar ambient UV, enhanced UV-A, enhanced UV-B, and enhanced UVA + B) (as subplots). Application of different levels of PAR and UV was started two weeks after transferring the pots to the outdoor. The rose geranium plants (5 pots per each treatment) received the individual treatments for three months. At the beginning of treatments, photoperiod was 14:30 h/9:30 h with day/night. In order to evaluate whether UV irradiation influences on rose geranium depending on PAR intensity, plants were exposed to two different PAR intensities. Average of ambient PAR intensity (High PAR) was at 1500 mmol/m²/s during midday on sunny days. In reduced PAR treatment (low PAR), plants received the PAR intensity of 800 mmol/m²/s by shading (corresponded to 53% of natural PAR intensity).

In enhanced UV-B and UV-A plots, the supplementary UV-B and UV-A treatments were applied in late June until the full flowering phase in late September. Plants were artificially subjected to UV-A and UV-B radiations via UV-A lamps (Q panel UV-A 340 40 W, 120 cm lamps, Q Panel Inc. Cleveland, OH, USA) and UV-B lamps (Q panel UV-B 313 40 W, 120 cm lamps, Q Panel Inc. Cleveland, OH, USA), respectively. In order to reduce shading of fluorescent lamps and metal frames, the experimental plots were placed in orientation of east-west. The lamps were fixed on frameworks at the northern margin of plots and UV radiation was reflected on the plants. The lamps were placed in steel frames with a distance of 25 cm between lamps at a distance of about 50 cm with plants. This distance was maintained steady until the experiment was virtually over. In the UV-A and UV-B plots, plants received ambient plus supplemental levels of UV-A and UV-B, respectively. Enhanced UV treatments was given to the plants at midday (11:00–14:00 h) by electric timer. The UV lamps were wrapped in cellulose diacetate foil to block residual UV-C irradiation (< 280 nm). In ambient UV plots, plants exposed to ambient rates of solar UV radiation.

2.3. Evaluation of biomass and yield attributes

At the full flowering stage, biomass and yield attributes including plant height, leaf length, leaf width, fresh and dry weight of plant aerial part and dry weight of leaf were measured. Plants were harvested and instantly weighed (recorded

fresh weight), followed by 72 h of oven drying at 50°C and reweighing (recoded dry weight). The oil-bearing tissue of the plant (leaves) were detached from the woody parts, weighed and noted as the leaf dry weight of *P. graveolens*.

2.4. Total phenol content

The total phenol content was assayed by Folin-Ciocalteu reagent method (McDonald et al., 2001). First, 1 g of dried leaves was ground and extracted in 10 mL of methanol (80%) and incubated at 20–25°C for 48 h in dark. After filtering the extract, 0.25 mL of extract, 0.25 mL Folin Ciocalteu reagent, and 2 mL Na₂CO₃ (1 M) were mixed well and left to stand at room temperature for 15 min. Finally, the absorbance of all samples was recorded using a spectrophotometer at 765 nm. All samples were analysed three times with the total phenol concentration measured based on the Gallic acid standard curve prepared using 0, 50, 100, 150, 250, and 500 mg/L solutions. The total phenol content was expressed in Gallic acid equivalent (mg GAE/g dry weight).

2.5. Total flavonoid content

The total flavonoid content was determined using the colorimetric method of aluminum chloride (Quettier-Deleu et al., 2000). Fifty µL of the standard or extract, 400 µL of 2% AlCl₃, and 1200 µL of 5% CH₃COOK were mixed and kept at 22°C for 40 min at 37°C. The absorbance of samples was read at 415 nm using a spectrophotometer. The standard curve was prepared using solution of rutin with concentrations of 0, 50, 100, 150, 250 and 500 mg/L and the total flavonoid content was expressed in rutin equivalent (mg rutin/100 g dry weight). The number of repetitions was three.

2.6. Antioxidant activity

The antioxidant capacity of leaf extracts was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay as in Choi et al. (2002). Various solutions of each extract (dosage range of 0.01-1.0 mg/mL) were prepared in methanol. Afterwards, 1.0 mL of DPPH solution 3.0×10^{-4} M and 2.5 mL of the extract were vigorously mixed and incubated in the dark at 20°C for 30 min. The absorbance of samples was read at 517 nm using a spectrophotometer. Then, the absorbance of a blank containing 2.5 mL of plant extract and 1.0 mL methanol was also read. Three repetitions were measured for each sample. Finally, the radical scavenging activity of each sample was expressed based on IC₅₀ (The half maximal inhibitory concentration).

2.7. Essential oil isolation

Twenty-five g of dried leaves were separately subjected to hydro-distillation using a Clevenger-type apparatus over 3 h. The essential oil was gathered in a glass vial, then stored at 4°C until GC-FID and GC-MS analyses. The content of essential oils obtained from each treatment was calculated from three sequential repetitions and expressed in mg per 100 g dry weight (w/w) (Taheri-Garavand et al., 2021).

2.8. GC–MS and GC–FID analyses

GC-MS and GC-FID analyses of essential oils were performed according to the work of Mumivand et al. (2021c). For GC-MS analysis, a Shimadzu gas chromatograph coupled with a mass spectrometer was employed. Essential oil constituents were separated through a fused-silica capillary column of 30 m × 0.22 mm i.d. coated with 0.25 µm film of BP-5 (Shimadzu). Carrier gas: ultra-pure helium; injector and interface temperatures: 280 and 260°C, respectively; ionization voltage: 70 eV; split ratio: 1/20; ion source and transfer-line temperatures: 250°C; mass spectrum: 35–450 amu. The oven temperature was adjusted to rise from 50 to 280°C at a rate of 5°C/min and finally held isothermally for 15 min.

The GC-FID analysis of essential oils was done through a Thermoquest Finnigan apparatus equipped with FID detector and a fused-silica capillary column of BP-5 with 30 m l. × 0.25 mm i.d. and film thickness of 0.25 mm. The temperature of oven was similar to GC-MS; detector and injector temperatures: 300°C and 250°C, respectively; carrier gas: helium; flow rate: 1 ml/min; split ratio: 1/20. Retention indices of each oil constituent were calculated using a homologous series of *n*-alkanes (C5-C25) injected to HP-5MS column in the similar circumstance. Constituents of essential oils were identified by comparing (1) the retention time with those from available authentic standards, (2) the mass spectra with those of the internal library, and (3) the retention indices (KI) with those presented in the literature (Adams, 2007). Relative area percentage obtained by GC–FID for each peak was used for quantification, without considering response factors (Mumivand et al., 2021c).

2.9. Data analysis

The mean of data obtained from the 2-years study was subjected to variance analysis according to the experimental design by SAS 9.1.3 (SAS Institute Inc.),

and mean comparison was done using Duncan's multiple range test at $P \leq 0.05$.

3. Results

3.1. Biomass and yield attributes

Results of the present study revealed that the length and width of leaf were significantly increased by lowering PAR intensity. The highest leaf length (5.37 cm) and leaf width (7.86 cm) were observed in plants exposed to low PAR intensity. On the contrary, increasing PAR intensity significantly improved the leaf dry weight. Leaf dry weight was increased by 21.95% when plants were exposed to high PAR compared with low PAR. (Table 1).

Table 1

The effect of different levels of PAR intensity on yield attributes, total phenol and essential oil content and yield of *P. graveolens*.

PAR	Leaf length (cm)	Leaf width (cm)	Leaf dry weight (g)	Total phenol (mg Gallic acid/g dry weight)	Essential oil content (%)	Essential oil yield (g/plant)
High PAR	3.88 ^b	5.69 ^b	69.78 ^a	68.54 ^a	0.431 ^a	0.298 ^a
Low PAR	5.37 ^a	7.86 ^a	57.22 ^b	55.91 ^b	0.336 ^b	0.187 ^b
Means with similar letters in each column, based on Duncan test at a 0.05% probability level, do not differ significantly.						

The length and width of leaf were significantly decreased by UV-B treatment in comparison with ambient UV. However, we did not find any effects of the UV-A irradiation on leaf dimensions. The highest leaf length and leaf width was observed in ambient UV treatment (5.48 and 7.94 cm, respectively). Furthermore, leaf dry weight was significantly declined by UV-B treatment. The highest leaf dry weight (69.4 g) was obtained in ambient UV treatment (Table 2).

Table 2

The effect of UV irradiation on yield attributes, total phenol and essential oil content and yield of *P. graveolens*

UV irradiation	Leaf length (cm)	Leaf width (cm)	Leaf dry weight (g)	Total phenol (mg Gallic acid/g dry weight)	Essential oil content (%)	Essential oil yield (g/plant)
Ambient UV	5.48 ^a	7.94 ^a	69.41 ^a	55.52 ^c	0.283 ^b	0.192 ^b
Enhanced UVA	5.4 ^a	7.57 ^a	67.22 ^a	55.23 ^c	0.321 ^b	0.225 ^b
Enhanced UVB	3.82 ^b	5.85 ^b	57.55 ^b	66.88 ^b	0.467 ^a	0.277 ^a
Enhanced UVA + B	3.81 ^b	5.73 ^b	59.83 ^b	71.28 ^a	0.464 ^a	0.276 ^a
Means with similar letters in each column, based on Duncan test at a 0.05% probability level, do not differ significantly.						

The interaction effect of UV radiation * PAR intensity was significant on height of plant and fresh and dry weight of aerial part. The highest plant height (51.08 cm) and fresh weight of aerial part (579 g) was observed in plants exposed to low PAR intensity and UV-A irradiation. On the contrary, the highest dry weight was obtained when plants were subjected to enhanced UV-A irradiation and high PAR intensity (Table 3).

Table 3

The interaction effect of PAR intensity and UV irradiation on yield attributes, total flavonoid and antioxidant capacity of *P. graveolens*

PAR intensity	UV irradiation	Plant height (cm)	plant fresh weight (g)	plant dry weight (g)	Total flavonoids (mg Routin/g dry weight)	Antioxidants capacity (µg/mL)
High PAR	Ambient UV	38.67 ^{bc}	512 ^c	95.94 ^{ab}	19.69 ^c	0.082 ^b
	Enhanced UVA	40 ^b	544 ^b	101.54 ^a	20.2 ^c	0.066 ^{bc}
	Enhanced UVB	30.67 ^d	437 ^d	90.91 ^b	23.77 ^b	0.052 ^c
	Enhanced UVA + B	32.5 ^d	441 ^d	95.85 ^{ab}	28.17 ^a	0.051 ^c
Low PAR	Ambient UV	46.67 ^a	572 ^a	91.85 ^b	13.97 ^d	0.16 ^a
	Enhanced UVA	51.08 ^a	579 ^a	95.58 ^{ab}	15.08 ^d	0.143 ^a
	Enhanced UVB	32.07 ^d	314 ^f	58.15 ^c	24.78 ^b	0.083 ^b
	Enhanced UVA + B	33.67 ^{cd}	384 ^e	61.98 ^c	26.1 ^{ab}	0.068 ^{bc}

Means with similar letters in each column, based on Duncan test at a 0.05% probability level, do not differ significantly.

3.2. Total phenol content, total flavonoid content and antioxidant activity

The concentration of total phenolics was significantly enhanced following the increasing PAR intensity. Total phenol content was stimulated by 22.59% in the high PAR treatment as compared with the low PAR (Table 1). Furthermore, UV-B irradiation strongly increased the total phenol content in comparison with ambient UV treatment. While, we did not observe any effects of the UV-A irradiation on total phenol content. Exposure of plants to the UV-A + B irradiation resulted in the highest total phenol content (71.28 mg Gallic acid per g of dry weight) (Table 2). Interaction of PAR intensity * UV treatments was significant for the total flavonoid content. The highest amount of total flavonoid content (28.17 mg rutin per g dry weight) was obtained in high PAR intensity and UV-A + B treatment. The results indicated that the antioxidant capacity of plants was strongly affected by the interaction of PAR intensity * UV treatments. The lowest IC₅₀ value (0.05 µg/mL) was obtained when plants were exposed to high PAR intensity and UV-A + B irradiation (Table 3). The lesser is the IC₅₀, the greater is the antioxidant capacity of the sample (Mumivand et al., 2021b).

3.4. Essential oil content and yield

The results revealed that under high PAR intensity, the essential oil of content and yield were increased by 28.27 and 59.35%, respectively, when compared with the low PAR intensity. The highest essential oil content (0.431%) and yield (0.298 g/plant) were obtained when plants were exposed to high PAR intensity (Table 1). The essential oil content and yield under UV-B irradiation illustrated a significant increment, so that the highest values of essential oil content and yield (0.467% and 0.277 g/plant, respectively) were obtained under enhanced UV-B treatment (Table 2).

3.5. Essential oil composition

In the present study, 34 constituents were identified in the essential oil of *P. graveolens* using GC-MS and GC-FID analysis (Table 4). Citronellol (up to 46.28%) and geraniol (up to 18.5%) were detected as the most abundant components of essential oil. Other notable constituents were neral (up to 4.3%), geranyl formate (up to 3.6%), *E*-rose oxide (up to 2.4%), citronellyl formate (up to 2%), β -elemene (up to 1.6%) and caryophyllene oxide (up to 1.5%). The mean comparison revealed that the percentage of geraniol in essential oil was enhanced by increasing the PAR intensity. The highest amount of geraniol (18.19%) was obtained in plants exposed to high PAR intensity. The citronellol/geraniol (C/G) ratio was significantly reduced from 2.49 in low PAR intensity to 2.38 in high PAR intensity (Fig. 1). UV-B irradiation significantly induced *E*-rose oxide production, so that the highest amount of *E*-rose oxide (2.37%) was noted under enriched UV-B treatment. The highest total amount of geraniol was observed in plants treated by both UV-A and UV-B. On the contrary, C/G ratio was significantly declined with UV-B irradiation. The highest C/G ratio was noted following ambient UVA treatment (Fig. 2).

Table 4
Essential oil constituents of *P. graveolens*

NO	Oil Constituents	RI ^a	LIT RI ^b	ID ^c
1	<i>p</i> -Cymene	1018.44	1020	RI, MS
2	Limonene	1020.15	1024	Std
3	β -Eocimene	1042.37	1044	RI, MS
4	Linalool	1090.95	1095	Std
5	<i>Z</i> -Rose Oxide	1102.55	1106	RI, MS
6	<i>E</i> -Rose Oxide	1120.76	1122	RI, MS
7	β -Citronellal	1142.86	1148	RI, MS
8	<i>Z</i> - <i>p</i> -Menthan-2-one	1190.3	1194	RI, MS
9	Citronellol	1122	1223	Std
10	Neral	130.32	1235	RI, MS
11	Geraniol	1249.64	1249	Std
12	Geranial	1261.65	1264	RI, MS
13	Neryl formate	1275.27	1280	RI, MS
14	Citronellyl formate	1270.88	1271	RI, MS
15	Geranyl formate	1288.64	1298	RI, MS
16	Methyl geranate	1320.45	1322	RI, MS
17	2-Phenyl ethyl propanoate	1350.65	1351	RI, MS
18	α -Cubebene	1352.64	1345	RI, MS
19	Citronellyl acetate	1353.96	1350	RI, MS
20	α -Copaene	1370.34	1374	RI, MS
21	β -Elemene	1385.65	1389	Std
22	β -Bourbonene	1386.7	1387	RI, MS
23	<i>E</i> -Caryophyllene	1409.64	1417	Std
24	Aromadendrene	1436.5	1439	RI, MS
25	Citronellyl propanoate	1447.65	1444	RI, MS
26	Germacrene-D	1482.5	1484	Std
27	Leden	1493.8	1496	RI, MS
28	Citronellyl butyrate	1513.5	1517	RI, MS
29	<i>E</i> -Calamenene	1522.4	1528	RI, MS
30	Geranyl butyrate	1558.42	1559	RI, MS
31	Spathulenol	1574.62	1577	Std

^a RI, linear retention indices on HP-5 column, experimentally determined using homologue series of n-alkanes.

^b Relative retention indices taken from Adams.

^c Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 05; RI: by comparison of retention index with those reported in literature; Std: by comparison of the retention time and mass spectrum of available authentic standard.

NO	Oil Constituents	RI ^a	LIT RI ^b	ID ^c
32	Caryophyllene oxide	1581.43	1582	Std
33	Phenyl ethyl tyglate	1583.54	1584	RI, MS
34	α -Bisabolol	1682.3	1685	RI, MS
^a RI, linear retention indices on HP-5 column, experimentally determined using homologue series of n-alkanes.				
^b Relative retention indices taken from Adams.				
^c Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 05; RI: by comparison of retention index with those reported in literature; Std: by comparison of the retention time and mass spectrum of available authentic standard.				

By comparing the mean interaction of UV irradiation * PAR intensity, the highest percentage of citronellol (46.28%) as the main essential oil ingredient was obtained under both low PAR intensity and enhanced UVA treatment. The highest total amounts of neral (4.32%) and citronellyl formate (2%) were found when plant were exposed to both low PAR intensity and enhanced UV-B. During subsequent exposure to high PAR intensity and UV-A, plants showed the highest percentage of geranyl formate (3.65%). Furthermore, the highest percentages of β -elemene (1.63%) and caryophyllene oxide (1.5%) were noted under low PAR intensity and ambient UV (Fig. 3).

3.6. Correlation between traits

The essential oil content showed a negative correlation with plant height (-0.74**) and plant fresh weight (-0.61**). But, it was positively correlated with total phenol (0.82**) and flavonoid (0.79**) contents. There was a high positive correlation between essential oil yield and essential oil content (0.88**). The correlation coefficient between antioxidant activity with essential oil content and yield, total phenol, flavonoid and C/G ratio were calculated as 0.82**, 0.79**, 0.75** and 0.75**, 0.67**, respectively. The percentage of geraniol in essential oil was negatively correlated with the plant height (-0.66**), plant fresh weight (-0.60**), plant dry weight (-0.55**) and leaf dry weight (-0.40*). Finally, C/G ratio correlation with essential oil content (0.87**), essential oil yield (0.69**), phenol (0.51*) and flavonoid (0.74**) was positive (Table 5).

Table 5

	Height	PFW	PDW	LDW	Oil content	Oil yield	Phenol	Flavonoid	DPPH	Citronellol	Geraniol	C/G ratio
Height	1											
PFW ^a	0.78**	1										
PDW ^b	0.39	0.81**	1									
LDW ^c	0.21	0.68**	0.94**	1								
Oil content	-0.74**	-0.61**	-0.19	-0.12	1							
Oil yield	-0.59**	-0.25	0.26	0.36	0.88**	1						
Phenol	-0.72**	-0.45*	0.013	0.12	0.82**	0.83**	1					
Flavonoid	-0.82**	-0.77**	-0.41*	-0.28	0.79**	0.62**	0.79**	1				
DPPH	-0.77**	-0.55**	-0.12	0.07	0.75**	0.75**	0.79**	0.82**	1			
Citronellol	-0.07	0.12	0.36	0.28	0.12	0.21	0.14	0.15	0.05	1		
Geraniol	-0.40*	-0.55**	-0.60**	-0.66**	0.07	-0.23	0.02	0.22	-0.05	-0.02	1	
C/G ratio	-0.79**	-0.77**	-0.49*	-0.39	0.74**	0.51*	0.69**	0.87**	0.67**	-0.01	0.31	1
The correlation coefficients between yield attributes, antioxidant activity, phenol, flavonoid, essential oil content and yield and major compounds of essential oil of <i>P. graveolens</i> .												
a) Plant fresh weight												
b) Plant dry weight												
c) Leaf dry weight												

4- Discussion

In the present research, we studied the response of *P. graveolens* to UV and PAR in terms of growth, yield, phenol and flavonoid contents, antioxidant capacity and essential oil composition. Our results proof that high PAR intensity declined the length and width of leaf and the height of plant, resulting in formation of dense and dwarf plants. Corresponding findings have been previously reported for *Dianthus caryophyllus* L. (Hlatshwayo and Wahome, 2010), *Hordeum vulgare* L. (Klem et al., 2012), *Pelargonium zonale* (L.) L'Hér. ex Aiton (Vidović et al., 2015) *Rosmarinus officinalis* L. (Raffo et al., 2020), which produced lower leaf area and plant height in the presence of high PAR intensity. However, similar to our results, plant dry weight were positively affected by PAR induction. As reported in previous studies, the high PAR intensity generally increased plant dry matter, and this effect is probably linked to the induced photosynthetic activity resulting in increased carbon fixation (Raffo et al., 2020). On the other hand, increasing leaf area in plants exposed to low PAR intensity can be interpreted as a kind of adaptation strategy to maximize the capture rate of radiant energy (Givnish, 1998).

Results showed different growth responses between plants exposed to enriched UV-A and enriched UV-B, as enriched UV-A radiation was more compatible and favorable for *P. graveolens*. Enriched UV-B irradiation was associated with reduced plant height, leaf expansion and fresh and dry weight of aerial part. These findings are in accordance with the earlier reported studies on UV-A and UV-B effects (Shayganfar et al., 2018; Mumivand et al., 2021d). Reduction in growth and yield of plants exposed to enhanced UV-B irradiation is mostly due to the negative effects of high UV-B on photosynthesis (especially photochemical efficiency of PSII) and chloroplast ultrastructure, DNA damage as well as the indole acetic acid photo-oxidative destruction (Klem et al., 2012; Matsuura, et al., 2013; Hui et al., 2014). Moreover, reduction of growth and yield of plant with UV-B irradiation was higher under low PAR intensity than high PAR intensity, confirming that UV-B irradiation and high PAR synergistically motivate defense systems in plant. As a support to our results, it has been previously demonstrated that blue light may ameliorate UV-B adverse effects (Vidović et al., 2015). Corresponding findings have been previously published for two barley varieties, where negative influences of enriched UV-B radiation on morphology and growth of plant were largely alleviated by high PAR intensity (Klem et al., 2012). As noted in that study, high PAR intensity prevented negative UV-B influence on the extent of light absorption, carboxylation activity as well as photochemical efficiency.

Increasing evidence suggests that plants exposure to excessive amounts of UV-B radiation quite often leads to overproduction of reactive oxygen species (ROS) in plant cells, due to impairing the photosynthetic machinery (Shayganfar et al., 2018). Plant cells are usually preserved against the adverse influences of ROS by a concerted action of both enzymatic and non-enzymatic antioxidant constituents. The antioxidants compounds act synergistically to scavenge increased level of ROS and mitigate stress (Mumivand et al., 2023). Previous studies reported that UV-B promotes the activities of antioxidant compounds, such as α -tocopherol, flavonoids, carotenoids and phenolics, which play important roles as defenses against ROS (Klem et al., 2012; Müller et al., 2013; Müller et al., 2015). As noted in the present study, the amount of total phenols and flavonoids and antioxidant activity of *P. graveolens* strongly increased with the UV-B radiation. Of all groups of secondary metabolites, phenolic compounds, especially flavonoids, are considered as the most related for UV-B defense. Therefore, it can be concluded that plant produces higher amount of these secondary metabolites to counteract the effects of UV-B irradiation. On the contrary, UV-A radiation did neither significantly influence total phenol and flavonoid contents nor antioxidant activity. Results demonstrated that high PAR intensity also induced the contents of total phenol and flavonoids in plant irrespective of UV treatment. Moreover, the combination of high PAR intensity and enhanced UV-B led, not only to further increases in total flavonoid content, but also in the antioxidant capacity. Therefore, it was found that PAR and UV-B had an increasable influence on the accumulation of flavonoids as UV-absorbing compounds

It is commonly acknowledged that epidermally located flavonoids, as photoprotective pigments, reduce the penetration of short wavelengths of solar radiation (280–450 nm) into leaves (Burchard et al., 2008), thus efficiently protect the photosynthetic apparatus from high PAR intensity and UV-B irradiation and prevent photo-oxidative damage (Müller et al., 2013). It has also been reported that flavonoids are endogenous regulators of transport and oxidation rates of indole acetic acid (Jansen, 2002). Therefore, further increase in flavonoid accumulation by UV-B and PAR irradiance could attribute to their roles as defense and signal compounds for protecting the plants. Furthermore, our findings demonstrated the negative relationship between flavonoids concentration and growth and yield of the plant.

We have shown that both high PAR and enhanced UV-B particularly increased the essential oil content and yield of *P. graveolens*. In accordance with this observation, an increased essential oil content of the plants exposed to higher PAR intensity has been reported in *Aeollanthus suaveolens* Mart. ex Spreng. (Barbosa et al., 2021), *R. officinalis* (Raffo et al., 2020), *Eclipta alba* (L.) Hassk. (Rai and Agrawal, 2020), and has been commonly ascribed to the induced photosynthetic activity and corresponding rate of basic metabolites. The higher accumulation of plant secondary metabolites in response to high PAR intensity could also be explained by the ecological functions of these compounds as a part of a plant defense mechanism against high light intensity (Raffo et al., 2020). Differently from what observed in this research, shading of solar radiation has been associated to the increased essential oil content of some species, due to the protection conferred by shading against the very intense radiation stress (Degani et al., 2016; Mousavinik et al., 2016).

The essential oil yield of oil-bearing plants is thoroughly dependent on the plant biomass and percentage of essential oil (Mumivand et al., 2021b). The extent of increasing the essential oil content due to enriched UV-B irradiation was higher than that of decreasing the leaf dry weight. For this reason, the essential oil yield of the plant was promoted by enhanced UV-B treatment. Thus, the induced amount of essential oil content is the dominant factor in determining an increased essential oil yield in *P. graveolens*. Results showing UV-B-induced incitement of production of essential oil is in accordance with observations of previous reports on other medicinal plants such as *Acorus calamus* L. (Kumari et al., 2009), *Ocimum sanctum* L. (Kumari and Agrawal, 2011), *Thymus* spp. (Shayganfar et al., 2018) and *alba* (Rai and Agrawal, 2020). Rai and Agrawal (2020) reported that the content of *E. alba* essential oil was increased under continuous UV-B irradiation, while reduced under intermittent UVB irradiation. Kumari and Agrawal (2011) have concluded that exposure of UV-B is a substantial need for the development and filling of the oil glands or glandular trichomes in *O. sanctum*, resulting in the boosting of the essential oil secretion. One of the roles of essential oils is participation in defensive mechanisms in response to environmental stresses, and their production is associated with abiotic factors such as nutrient deficiency, salt stress, water scarcity, temperature, light intensity, and UV irradiation. (Wang et al., 2019; Mumivand et al., 2021a). Thus, increasing the essential oil content of rose-geranium to induce plant tolerance to UV-B irradiation and its oxidative stress seems reasonable. In this study, enhanced UV-A irradiation made no change in essential oil content and yield of plant. This result was inconsistent with finding reported by Mumivand et al., (2021d) in thyme, who mentioned a positive effect of enhanced UV-A irradiation on the essential oil production of the studied species. These differences in the responses of the plants could be due to factors including the inherent characteristics of the species, plant origin, climatic conditions, extraction methods, storage conditions, and dose and duration of UV-B irradiation (Morshedloo et al., 2021; Mumivand et al., 2021d).

A change in essential oil composition under both UV-B irradiance and PAR intensity has been demonstrated in a majority of papers, although in some cases it remains constant. In the present study, the data show that the UV irradiation and PAR intensity significantly affected the essential oil constituents of *P. graveolens*. High PAR intensity increased the amount of geraniol in essential oil, leading to a slight reduction of C/G ratio. Furthermore, significant decrease in C/G ratio was observed in the enriched UV-B irradiation in comparison with ambient UV. The C/G ratio is a key index characterizing the quality of the essential oil in rose-geranium for the perfume industry

(Saxena et al., 2008). C/G ratio of 1:1–3:1 give the economic value to rose-geranium essential oil (Verma et al., 2010). However, it believes that essential oils with a C/G ratio higher than 3:1 is have poor quality for the perfume industry (Peterson et al., 2006). Considering that the lower C/G ratio is the most important indicator of the high economic value of rose-geranium essential oil, reducing C/G ratio under enriched UV-B radiation and/or high PAR is likely to be favorable.

Significant increase was observed in the percentage of geraniol under enriched UV-B treatment. While, under low PAR intensity, neral and citronellyl formate showed increment in plants exposed to enhanced UV-B. On the other hand, under high PAR intensity, caryophyllene oxide showed reduction following enhanced UV-B exposure. Citronellol and β -elemene both found to be declined in *P. graveolens* essential oil when plants were exposed to enriched UV-B. On the contrary, under low PAR intensity, enhanced UV-A radiation was positive for high citronellol content. It is hypothesized that such modification in secondary metabolites biosynthesis is necessary for plant adaptation to biotic and abiotic stresses such as PAR intensity and UV-B radiation (Manukyan, 2013). The effect of PAR and UV-B on essential oil components is likely due to its effects on modulating the expression of genes involved in phenylpropanoids and terpenoids biosynthetic pathways (Kumari et al., 2009). A slight change in enzymatic activity may lead to an increase or decrease in certain compounds and make a change in the composition of essential oil (Figueiredo et al., 2008). Citronellol and geraniol, as the major constituents of *P. graveolens* essential oil, are alcoholic monoterpenes synthesized by the biosynthetic pathway of methylerythritol-4-phosphate (MEP) (Saxena et al., 2008; Verma et al., 2010). The precursor supplies (IPP and DMAPP) is a limiting factor in the biosynthesis of monoterpenes from the plastidial MEP pathway (Dolzhenko et al., 2010). Considering the fact that monoterpenes are synthesized in plastids, any chloroplast damage induced by UV-B and/or high PAR may lead to change in monoterpene biosynthesis. It has been shown that UV-B altered expression of genes involved in essential oil biosynthesis in peppermint, subsequently made change in essential oil composition (Dolzhenko et al., 2010; Alagupalamuthirsolai et al., 2019). Many publications report the change in essential oil composition under UV-B exposure or PAR intensity. For example, in lemongrass, UV-B exposure increased the essential oil content. Furthermore, the proportion of *Z*-citral, geraniol formate, polyol, linalyl formate was also significantly changed following UV-B treatment (Kumari et al., 2009). Rai and Agrawal (2020) also reported significant increment in some medicinally important constituents of *E. alba* essential oil such as α -terpineol, δ -cadinene, methyl linoleate, linolenic acid and myristic acid amide exposed to continuous UV-B irradiation. The findings obtained from the study of Raffo et al., (2020) on *R. officinalis* demonstrated that the relative percentages of camphene, α -pinene, myrcene, 1,8-cineole, β -pinene, α -terpinene, β -caryophyllene and α -phellandrene enhanced in plants exposed to 50% sunlight, whereas camphor, 3-carene, terpinen-4-ol, borneol, verbenone, α -terpineol, and humulene showed lowered abundances in this treatment.

5. Conclusions

In this outdoor study, an attempt was made to elucidate whether high PAR intensity can minimize reduction in growth and yield of *P. graveolens* exposed to UV-B irradiation. High PAR intensity declined the length and width of leaf, the height of plant, as well as fresh weight of aerial parts, but increased dry weight of aerial parts. On the other hand, enhanced UV-B irradiation was associated with reduced plant height, leaf expansion, and fresh and dry weight of aerial parts. Negative effect of enhanced UV-B irradiation on yield was noticeably ameliorated by high PAR intensity. Based on data of yield and yield attributes, our hypothesis was confirmed. Enhanced UV-B-induced changes in biosynthesis of plants secondary metabolites could be regarded as an eventual adaptation to UV-B irradiance and should be considered as a central element along with tolerance. Both high PAR and enhanced UV-B prompt the accumulation of phenols, flavonoids and essential oil. Considerable changes were also registered not only for citronellol and geraniol as major components of the essential oil, but also for some minor compounds. Interestingly, C/G ratio was significantly declined under enhanced UV-B radiation and/or high PAR, giving the higher economic value to rose geranium essential oil. Indeed, high level of secondary metabolites production is economically significant in medicinal plants cultivation. A positive relationship between the high PAR intensity or enhanced UV-B irradiation with total phenol, flavonoids, antioxidant activity as well as properties and production of essential oil was observed. Overall, we conclude that PAR and UV-B radiation, as two important environmental factors, have positive effects on the quality and quantity of rose-geranium secondary metabolites.

Declarations

Guideline statement

The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations.

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Availability of data and materials

All the data generated or analyzed during the current study were included in the manuscript. The raw data is available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The plant materials used in the present study were *Pelargonium graveolens* cutting from a cultivated clone growing in the Medicinal Plants Garden of Lorestan University, Khoramabad, Iran. For experimental research on plants, including the collection of plant material, we comply with relevant institutional, national, and international guidelines and legislation. All procedures were conducted in accordance to the guidelines.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

Maryam Jadidi: Investigation, Writing original draft; Hasan Mumivand: Methodology, Project administration, Conceptualization, Writing–review & editing and planned the experiments; Abdollah Ehtesham Nia: Validation, Writing -Review & Editing; Alireza Shayganfar: Validation, Software and Formal analysis; Filippo Maggi: Data interpretation, Writing –review & editing.

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Figures

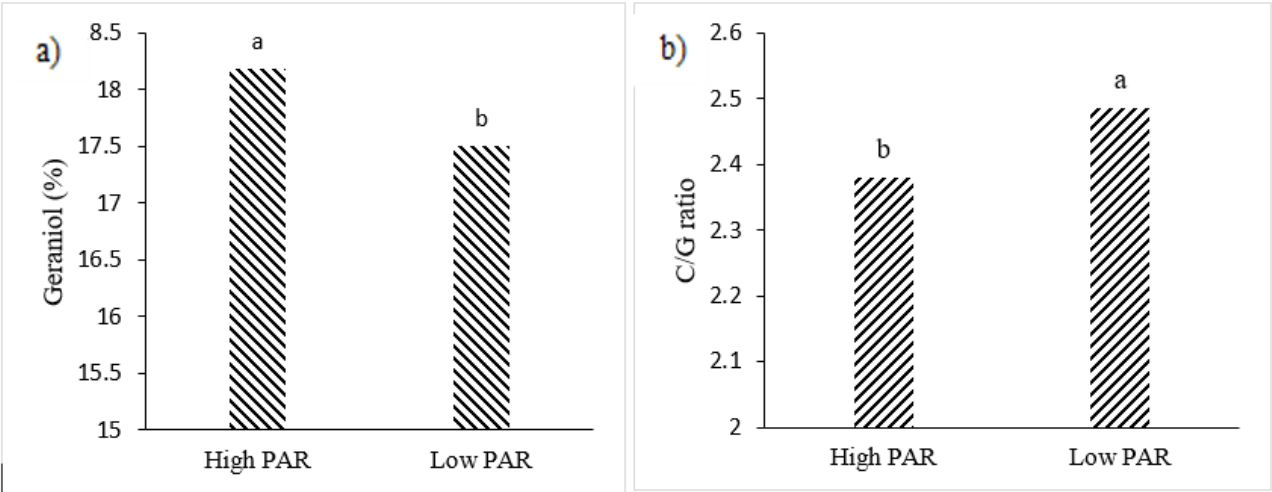


Figure 1
The effect of different levels of PAR intensity on (a) geraniol and (b) citronellol/geraniol ratio in the essential oil of *P. graveolens*.

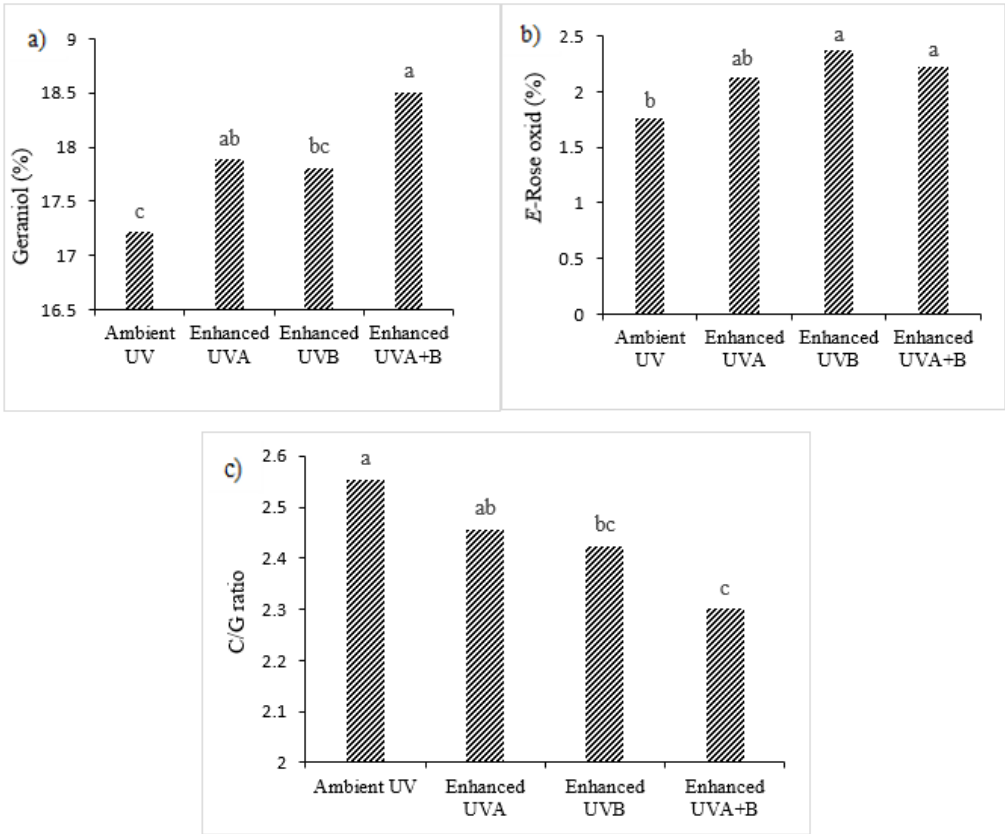


Figure 2
The effect of UV irradiation on (a) geraniol, (b) *E*-rose oxide and (c) citronellol/geraniol ratio in the essential oil of *P. graveolens*.

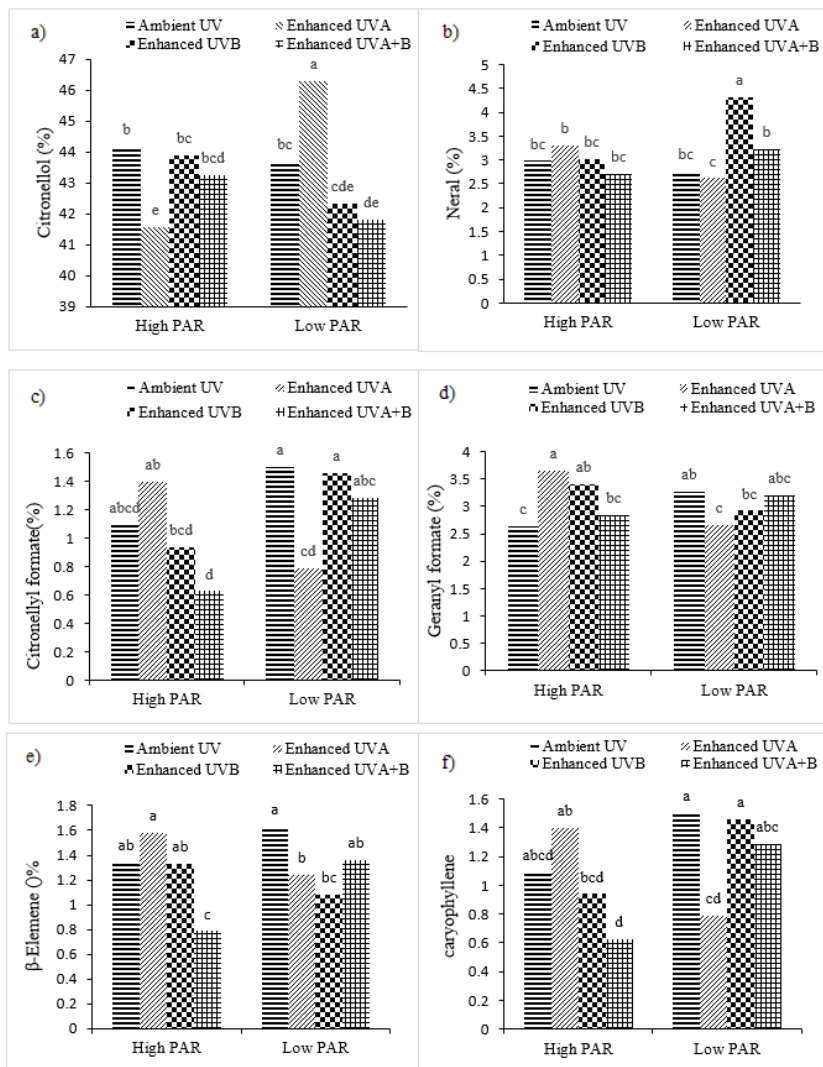


Figure 3

The interaction effect of PAR intensity and UV irradiation on (a) citronellol, (b) neral, (c) citronellyl formate, (d) geranyl formate, (e) β -Elementene and (f) caryophyllene oxide in the essential oil of *P. graveolens*. Ingredients of aromatic geranium essential oil