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The effects of Ultraviolet Radiation on some Growth Parameters, Pigments content and Antioxidant Enzymes Activity at early Developmental stages of *Dracocephalum moldavica*

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ABSTRACT: During the last decades, damaging of ozone layer results in the effect of solar UV radiation on the earth. The increase of ultraviolet radiation can have many direct or indirect deleterious effects on the living organisms. To study of enhanced ultraviolet radiation (under 320nm) effects we assayed some growth parameters, pigments and antioxidant activities of *Dracocephalum moldavica* as herbal medicine in 2-4 pair leaf stage. The plants were exposed to six doses of UV radiation (7, 15, 22, 30, 37, 51 $kjm^{-2}d^{-1}$). Data were analyzed using SPSS software and ANOVA test. Results of this experiment showed that UV doses decreased growth parameters of root and shoot (fresh and dry weight, length of root and shoot) and pigments contents (chlorophyll a, chlorophyll b and Total chlorophyll). Based on the results, there are significant increase in the amount of carotenoids and anthocyanins in high dosages of UV radiation. However the highest doses of UV enhanced activity of catalase and peroxidase but decreased superoxide dismutase and ascorbate peroxidase as antioxidant enzymes. Results suggested that UV radiation (under 320nm) could effect on growth parameters and enzymatic and non-enzymatic defence system in higher doses. The increased activity of antioxidant enzymes, anthocyanins and carotenoids in high doses of UV treatment shows the plant sensitivity levels in front of UV radiation and combination of enzymatic and non-enzymatic defence mechanisms, which decreased the effects of radiation damage in higher doses.

Keywords: *Dracocephalum moldavica*, UV, growth parameters, pigments, antioxidant enzymes

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INTRODUCTION

Among solar wide spread wavelengths, short ultraviolet (UV) irradiation is harmful against life. The stratospheric ozone layer filtered out UV irradiation as a shield and protects the earth against harmful ultraviolet (UV) radiation from the sun (Matsumi & Kawasaki, 2003; Piri *et al.,* 2011). During the past eighty years, stratospheric Ozone depletion, caused by greenhouse gas such as chlorofluorocarbons (CFCs) and NOx, methyl bromide (MeBr) and other industrial compounds containing halogens, results in increased levels of ultraviolet radiation reaching the Earth's surface (Kakani *et al.,* 2003; Krizek *et al.,* 1998).

Ultraviolet (UV) wavelength (200–400nm) has considerable biological impact on the living organisms, including human, animals and plants. This wavelength consists of UV-A (320- 400 nm), UV-B (2780-320nm) and UV-C (200-280 nm). The shortest UV waves (UV-B and UV-C) possess enough energy to damage and cause negative biological effects on growth, development, photosynthesis and reproduction by production of reactive oxygen species (ROS) and forming oxidative stress, that may decrease cell viability and cause cell death (Alexieva *et al.,* 2001; Danon & Gallois, 1998; Frohnmeyer & Staiger, 2003; Jansen, 2002; Kovacs & Keresztes, 2002; Procházková & Wilhelmová, 2007; Schreiner *et al.,* 2012; Takeuchi *et al.,* 2007; Zacchini & de Agazio, 2004). An antioxidant is a molecule that inhibits the oxidation of other molecules and has several properties (Parvaneh *et al.,* 2015: Mohammad *et al,* 2015; Haleh *et al.,* 2015).

Low doses of UV-B or C as environmental stress may activate enzymatic and non-enzymatic defence systems (Hideg *et al.,* 2013; Jansen, 2002; Katerova *et al.,* 2009; Zornitsa Katerova & Todorova, 2011; Lavola *et al.,* 2003; Loyall *et al.,* 2000; Rai *et al.,* 2011), but high doses of UV could induce the improvement of systems repairmen mechanisms (Frohnmeyer & Staiger, 2003) while in average doses, plants get reversible adaptations (Rogozhin *et al.,* 2000). However, plant sensitivity to UV radiation differs among species (Teramura, 1983) and varieties (Correia *et al.,* 1998; Reed *et al.,* 1992).

Dracocephalum moldavica belongs to the Lamiaceae family are one of the important medicinal plants. It is originated from the south of Siberia and the slopes of Himalaya. Essential oils of aerial parts have antioxidant activity and antiseptic, antibacterial, analgesic, anti-inflammatory, anticonvulsive; wound healing, sedative and antitumor properties. The amount of plant essential oils depends on the environmental factors (Dastmalchi *et al.,* 2007; Galambosi & Holm, 1989; R Omidbaigi *et al.,* 2009; Reza Omidbaigi *et al.,* 2010; Povilaityee & Venskutonis, 2000).

Despite extensive researches on crops, few studies on the impacts of ultraviolet radiation is done on medicinal plants (Kumari *et al.,* 2010; Singh *et al.,* 2011) especially Lamiaceae family. The purpose of presented study is to assess the effects of UV radiation (under 320nm) on some growth parameters, pigments content and antioxidant defence systems of *Dracocephalum moldavica*.

MATERIAL AND METHODS

A. Plant material and ultraviolation radiation treatment

The experiments were carried out in a greenhouse at the University of Tabriz. The seeds of *Dracocephalum moldavica* were sown into the pots containing 25% sand, 25% mineral soil mixture and 50% fertilizer. Dragonhead (*Dracocephalum moldavica*) plants were grown under controlled (light/dark) regime at 16/8 h at 24/20°C. By reaching the two - pair leaf stage, the pots transferred to an ultraviolet light chamber. The plants were treated by different doses of UV (Holland TL-D 15W Actinic BL) under 320 nm, during 13 days until appearing fourth leaf pair. (0 (control), 54, 108, 162, 216, 270 and 378) $kjm^{-2}d^{-1}$ per day for 13 consecutive days to reach the fourth stage Leaf. To analysis the enzymes activity and determination of pigments, fresh leaves were collected and frozen in liquid nitrogen after treatments and stored at -80°C.

B. Measurement of growth parameters

After measuring shoot height and root length of the harvested plants, shoot and root fresh and dry weight was measured using digital scale. The dry weight of the samples was measured after 72h in oven.

C. Evaluations of pigments content

Photosynthetic pigments (chlorophyll a, b, total chlorophyll, and total carotenoids) and anthocyanin contents were measured according to the method of spectrophotometric determination; Photosynthetic pigments were extracted from the 0.05 gr of fresh leaves of *Dracocephalum moldavica* with 2 ml of methanol for 24 hours at -4°C. Then the extract was centrifuged at 2500 g for 10 min. The contents of chlorophylls and carotenoids of the extract was calculated using the following formulas based on several recorded absorbance (Şükran *et al.*, 1998):

$C_a = 15.65\ A_{665} - 7.340\ A_{653}$
$C_b = 27.05\ A_{653} - 11.21\ A_{665}$
$C_{total} = C_a / C_b$
$C_{x+c} = 1000\ A_{470} - 2.860\ C_a - 129.2\ C_b/245$
Ca = Chlorophyll a, Cb = Chlorophyll b, C_{total} = Total Chlorophyll, Cx+c = Total carotenoids

D. Assay of anthocyanins content

For determination of anthocyanins content, samples were homogenized in acidified methanol (methanol (99): HCl (1) (v/v)) and incubated at room temperature for 24 hours in the dark. After centrifugation, absorption of the supernatant was measured at 530 and 657 nm using a spectrophotometer and the formula A530 – (0.25 × A657) was utilized to calculate the amount of anthocyanins.

E. Total protein content and antioxidant enzymes activities assays

An amount of 0.1 gr of samples was homogenized in ice-cold phosphate- buffered solution (PBS, 50 mM, pH = 7). Homogenates were centrifuged at 5000 g for 10 min at 4°C. The supernatants were used immediately for determination of the total soluble protein content (Bradford, 1976) as well as the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX).

Catalase (CAT) activity assay. CAT activity was assayed according to the methods of Chance and Maehly (1955). The activity of CAT was measured at 240 nm by following the decomposition of H_2O_2 for 3 min. The reaction mixture contained 2.5 ml potassium phosphate buffer (50 mM, pH=7), 1 ml H_2O_2 (10 mM) and 500 µl of enzyme extract. CAT specific activity was calculated using the extinction coefficient of 27 M^{-1} cm^{-1} for H_2O_2 and one unit of enzyme activity was considered as the amount of enzyme necessary for the reduction of 1 µM H_2O_2 per minute (Chance & Maehly, 1955).

Peroxidase (POD) activity assay. The activity of POD was determined by recording the increase in absorbance at 470 nm during polymerization of Guaiacol to tetraguaiacol for 3 minutes (Obinger *et al.*, 1997).

The reaction mixture (1 ml) encompassed 300 µl of guaiacol (4 mM), 350 µl of phosphate buffer (10 mM, pH=7), 300 µl of H_2O_2 (50 mM) and 50 µl of enzyme extract. The reaction was initiated by adding H_2O_2 to the reaction mixture and POD specific activity was calculated using the extinction coefficient of 26.6 mM^{-1} cm^{-1} in Guaiacol. One unit of POD activity was considered as the enzyme amount capable of oxidizing 1 µM Guaiacol to tetraguaiacol per minute.

Superoxide dismutase (SOD) activity assay. SOD activity was evaluated by determination of nitro-blue-tetrazolium (NBT) photoreduction inhibition by extracts (Winterbourn *et al.,* 1976). The reaction mixture (3 ml) contained 2.7 ml sodium phosphate solution (1 M, pH=7.8), 100 µl NBT (1.5 mM), 200 µl NaCN (0.3 mM), EDTA (1 M), 50 µl of riboflavin (0.12 mM) and 50 µl of enzyme extract. The mixtures were illuminated at light intensity of 5000 Lux for 12 minutes and the absorbance of the solutions was recorded at 560 nm. The amount of the enzyme that causing 50% protection of NBT photoreduction was considered as one unit and SOD activity expressed as U mg^{-1} protein.

Ascorbate Peroxidase (APX) activity assay. Ascorbate peroxidase (APX) activity was determined using the method of Boominathan and Doran. Oxidation of ascorbic acid was followed as a reduction in absorbance at 290 nm. The amount of enzyme protein required for the oxidation of 1 µmol ascorbic acid /min was defined as one unit (Boominathan & Doran, 2002).

F. Statistical analysis

All measurements were conducted with three replications and data were reported as mean ± SD. One-way analysis of variance was used to compare the means using SPSS ver.16 software. Duncan's test was used for paired comparisons at $p<0.05$. Microsoft excel 2013 software was used for the preparation of figures.

RESULTS AND DISCUSSION

A. Growth parameters

The results for growth parameters showed that UV radiation decreased dry weight of the shoots (Table 1). High dosages of UV radiation caused a significant increasing in fresh weight of root and shoot during treatments compared with the control plants. Based on the other research result, supplementary UV-B light in basil plants at the 3–4 leaf pair growth stage significantly increased fresh and dry biomass accumulation. Thus, the reduction in fresh and dry weight of shoot seems to have been due to a decrease in the amount of photosynthesis (Sakalauskaitė *et al.,* 2012). In the other work it was found a significant decrease in fresh and dry weight and dry matter content of common dandelion and purple coneflower seedlings after supplemental UV-C treatment (Castronuovo *et al.,* 2017). Also Ziska and Teramura, 1992; Barnes *et al.,* 1993, in rice; Teramura & Sullivan, 1994, in seeding loblolly pines (*Pinus taeda* L.) reported a reduction in dry matter weight and total biomass in response to supplemental UV-B. Kakani showed that in the 54% of studies, elevated UV-B radiation reduced the biomass of the plant while 35% of the researches reported no effect. Differences in the response of plants growth parameters to UV-B radiation can be due to different pathways of the effect and sensitivity of various plants associated with these waves (Kakani, Reddy *et al*., 2003; Mark *et al.,* 1996).

Based on the presented results, increase of shoot length and decrease of root length showed as non-significant results of high dosages of UV treatment in compare with the control. Earlier studies (Barnes *et al.,* 1990; Bilger *et al.,* 2001; Greenberg *et al.,* 1996; Kozłowska *et al.,* 2007; Warren *et al.,* 2003) reported marked reduction in plants height in exposed UV radiation. However, in the plants, response to UV-B varies among species (Barnes, Flint *et al.,* 1990; Cybulski & Peterjohn, 1999; Musil, 1995). Reduction of plant height by UV-B radiation was also observed in wheat, cucumber, wild oats, sunflower (Tevini & Teramura, 1989), soybean (Sullivan *et al.,* 1994) and rice (Barnes, Maggard *et al.,* 1993; Dai *et al.,* 1997). A photo oxidative degradation of the phytohormoneindole acetic acid might exposure to UV-B-induced reductions of plant height (Mark, Saile-Mark *et al.,* 1996).

Table 1: Means of growth parameters in *Dracocephalum moldavica* (≤ 320nm).

	Parameters					
	Shoot FW(	**Root FW(g)**	**Shoot DW(g)**	**Root DW(g)**	**Shoot length (cm)**	**Root height (cm)**
Control	0.16±0.02b	0.007±0.0012^{b}	0.203±0.015^{a}	-	11.05±0.75^{ab}	13.12±0.02^{a}
7 kjm^{-2}d^{-1}	0.13±0.02b	0.006±0.001^{b}	0.153±0.032^{a}	-	11.67±0.88^{ab}	13.00±0.58^{a}
15 kjm^{-2}d^{-1}	0.15±0.02b	0.008±0.0009^{b}	0.157±0.022^{a}	-	12.17±0.17^{ab}	12.21±0.12^{ab}
22 kjm^{-2}d^{-1}	0.16±0.01b	0.005±0.001^{b}	0.080±0.006^{b}	-	10.55±0.87^{b}	10.55±0.12^{b}
30 kjm^{-2}d^{-1}	0.26±0.03a	0.007±0003^{b}	0.066±0.011^{b}	-	12.21±0.12^{ab}	12.17±0.17^{ab}
37 kjm^{-2}d^{-1}	0.26±0.04a	0.009±0007^{ab}	0.053±0.009^{b}	-	13±0.58^{a}	11.67±0.88^{ab}
51 kjm^{-2}d^{-1}	0.33±0.03a	0.013±0.0032^{a}	0.087±0.007^{b}	-	13.12±0.02^{a}	11.05±0.75^{ab}

Results are means ±SE of 5 replicates. Means are significantly different at $p<0.05$

B. Pigments content

The results of the effect of ultraviolet radiation on photosynthetic pigments are shown in Table 2. In the presented study, a significant reduction in chlorophyll a, b and total chlorophyll contents. Reducing chlorophyll in low biomass can be one of the important indicators in the sensitivity of plants to ultraviolet radiation (Smith *et al.*, 2000). It seems that the decrease in chlorophyll content probably due to damage to the photosynthetic system in chloroplasts (Malanga *et al.*, 1997). It is known that UV-B and UV-C clearly reduced chlorophyll contents with destroying the structure of chloroplast, inhibiting the synthesis of chlorophylls and increasing the rate of chlorophylls degradations (Rahmatzadeh & Khara, 2007; Caldwell *et al.*, 1995; Du & Jin, 2000). In wheat plants showed that UV-C treatment increase chlorophyll a, b and carotenoids (Rahmatzadeh and Khara, 2007, Takeuchi and colleagues, in rice (Takeuchi *et al*., 2007) and Mahdavian and colleagues (Mahdavian *et al.*, 2008) in pepper showed a decreasing in contents of photosynthetic pigments under UV-C stress in the same line with our findings. Differently many reports suggested a marked reduction in total chlorophyll (Ambasht & Agrawal, 1998; Day & Vogelmann, 1995; Gitz III *et al.*, 2004; Ravindran *et al*., 2008; Skorska, 2000; Strid *et al.*, 1990). There were several effects on the photosynthesis machinery in response to UV radiation, including damage of plastoquinone, rubisco and chlorophylls. Due to high sensitivity of electron transport in PSII to UV light, it can inhibit electron transport through PSII and reduced fluorescence of chlorophyll a and intensities of the thermosluminescence Q and B bands (Ravindran *et al.*, 2008).

Moreover, the carotenoids value in plants exposed to low doses did not change, but the results showed a significant increase in the amount of carotenoids and anthocyanins in high dosages of UV radiation. The reduction of carotenoids in some plant species is an adaptation response (Hollósy, 2002) and protective function (Campos *et al.*, 1991; Rau *et al.*, 1991) to reduce the effects of UV rays. Increasing of carotenoids and anthocyanins in same level of UV radiation in the presented study showed an important stress level of UV radiation for *Dracocephalum moldavica*. Researches have indicated that anthocyanins and carotenoids could act as effective antioxidants (Sarma *et al.*, 1997), (Stahl & Sies, 2005). However anthocyanins are water soluble pigments (Mazza *et al.*, 2004) and reduced UV-B penetration and protect photosynthetic apparatus with binding to phytotoxins and cell division apparatus (Pal *et al.*, 1999; Winkel-Shirley, 2002).

Table 2: Pigments content and protein in *Dracocephalum moldavica* exposed on enhanced UV radiation (≤ 320nm).

	Parameters					
	Chlorophyll a (mg)	**Chlorophyll b (mg)**	**Total Chlorophyll (mg)**	**Carotenoids (mg)**	**Anthocyanin (mg)**	**Protein (mg)**
Control	321.10±7.01^{a}	154.7±7.7 ^{a}	475.76±9.01^{a}	22752.3±948.3^{b}	6.26±0.33^{d}	8.464±0.31^{b}
7 kjm^{-2}d^{-1}	325.50±8.54^{a}	141.4±6.2^{a}	466.92±7.79^{a}	22656.2±419.7^{b}	8.66±0.07^{ab}	10.299±0.63^{a}
15 kjm^{-2}d^{-1}	324.35±7.83^{a}	102.9±9.2^{b}	453.92±12.80^{ab}	22742.2±454.1^{b}	7.27±0.19^{c}	9.516±0.30^{ab}
22 kjm^{-2}d^{-1}	301.57±6.60^{b}	123.6±11.0^{ab}	425.18±17.52^{b}	29139.1±843.3^{b}	8.04±0.24^{bc}	9.584±0.41^{ab}
30 kjm^{-2}d^{-1}	300.01±5.46^{b}	111.4±9.8^{b}	375.08±13.36^{c}	27487.8±1299.4^{a}	9.29±0.58^{a}	8.518±0.69^{b}
37 kjm^{-2}d^{-1}	301.43±1.13^{b}	63.4±0.6^{c}	364.87±1.73^{c}	24369.6±86.1^{a}	9.44±0.33^{a}	9.107±0.52^{ab}
51 kjm^{-2}d^{-1}	113.22±1.54^{c}	61.0±10.5^{c}	181.76±11.52^{d}	27294.0±1237.4^{a}	9.69±0.30^{a}	9.262±0.40^{ab}

Results are means ±SE of 3 replicates. Means are significantly different at $p<0.05$

C. Antioxidant Enzymes and Protein Content

The stresses imposed by UV-light radiation can cause reactive oxygen species generation (ROS) such as O°2 and H_2O_2. H_2O_2 can easily diffuse through cell membranes, and it is deleterious to cellular components (Sarma, Sreelakshmi *et al.*, 1997; Sharma *et al.*, 2012). The results of ultraviolet radiation on antioxidant enzymes (SOD, POX, CAT and APX) and protein content are shown in Fig. 1 and Table 2 respectively. The results showed that superoxide dismutase activity decreased in higher doses of UV radiation. Ascorbate peroxidase also showed a significant reduction in treated plants compared to non-irradiated (control) plants while the results showed an increasing trend in the enzyme activity with increasing the UV dosages. The results of the peroxidase and catalase showed an increasing trend, but a significant increase was seen at 30, 37 and 51 kjm^{-2}d^{-1} of UV radiation. The amount of protein also significantly increased during the first half-hour (7 kjm^{-2}d^{-1}).

Catalase with peroxidase, are known to reduce the concentration of O_2^-, OH^- and H_2O_2 (Bowler *et al.*, 1992).

Increase in activities of peroxidases by UV-B radiation have been observed in several species including Cassia (Sheela Agarwal & Pandey, 2003), *Arabidopsis thaliana* (Rao *et al.*, 1996), cucumber (Krizek *et al.*, 1993), sugar beet (Panagopoulos *et al.*, 1990) and potato (Santos *et al.*, 2004). However, there are differences in antioxidant responses among species, genotypes of the same species (Hideg *et al.*, 2006; Xu *et al.*, 2008) and developmental stages (Lidon & Ramalho, 2011; Majer & Hideg, 2012). CAT activity in the peroxisomes by changing H_2O_2 into O_2 and APX activity in the cytosol, mitochondria, and chloroplasts detoxified generally synthesis of hydrogen peroxide (Asada, 2006; Foyer *et al.*, 1997) whereas peroxidase decomposes H_2O_2 by oxidation of co-substances. The effect of peroxidase in protection of membrane damage is well known (GASPART *et al.*, 1991) based on the enhanced levels of peroxidase activity in the presented results, it is shown the enzymatic mechanism against increasing of membrane damage in higher doses of UV irradiation.

In this study, in the same line with the other reports (A-H-Mackerness *et al.*, 1998; Kondo & Kawashima, 2000), SOD activity was decrease in response to the elevated levels of UV radiation. Decreasing in the SOD activity leads to O_2^-accretion and therefore could be responsible for chlorophyll diminution (Xu, Sullivan *et al.*, 2008). That was many reports contrast our results revealing increased SOD activity by UV-B radiation for example, in pea and wheat (Alexieva, Sergiev *et al.*, 2001), *Arabidopsis* (Rao & Ormrod, 1995), (Babu *et al.*, 2003) and rice (Dai, Yan *et al.*, 1997). In the other hand, there was not affected in barley (Mazza *et al.*, 1999), *Nicotiana plumbaginifolia* L. (Willekens *et al.*, 1994) and it was decreased in the sunflower cotyledon (Costa *et al.*, 2002). SOD converts superoxide radicals (O^{-2}) into H_2O_2 (Kondo & Kawashima, 2000). Inhibition of SOD activity by UV-B could lead to increases in O_2-content. H_2O_2 production was increased by high levels of UV-B in several studies conducted indoors (Alexieva, Sergiev *et al.*, 2001; Hideg, Rosenqvist *et al.*, 2006; Kalbina & Strid, 2006). In the magenta line of soybean solar UV-B radiation decreased the SOD activity and increased the CAT activity at 8 days (Xu, Sullivan *et al.*, 2008). In the other hand, based on the decreasing amount of chlorophylls in high dosages of UV exposure, inhibition of photosynthesis and consequences in decreasing water hydrolysis and O_2 generations led us to understand the reason of decreasing of SOD activity in same doses of UV radiation.

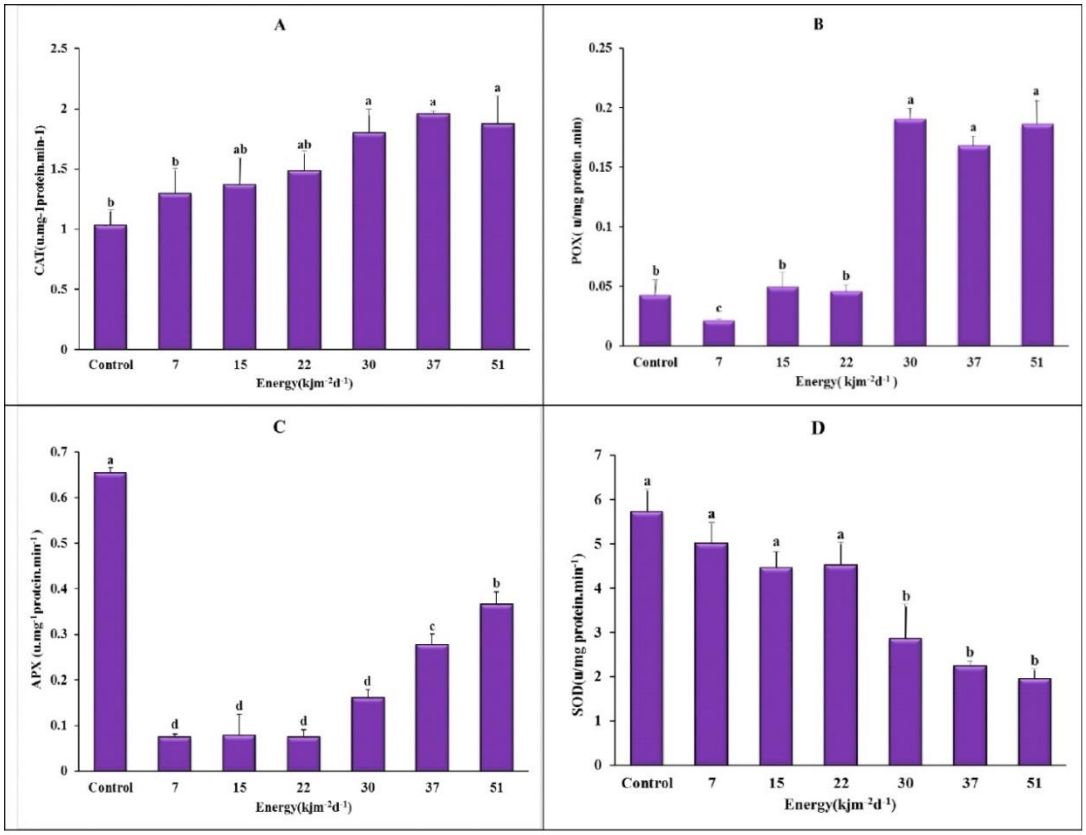

Fig. 1. The effects of enhanced UV radiation (UV≤ 320nm) on the antioxidant enzymes of D. moldavica. A: CAT, B: POD, C: SOD, D: APX, SOD, superoxide dismutase; APX, ascorbate peroxidase, catalase; POD, peroxidase. The bars with different letters were significantly different from each other (P≤ 0.05). Values were means ± SE (n = 3).

Antioxidative enzymes like catalase, peroxidase, and ascorbate peroxidase are involved in ascorbate-glutathione cycle for detoxification of excess H_2O_2 produced under stresses (Noctor & Foyer, 1998). Presented results is supported with some other studies reporting induction of the activities of these enzymes under UV-B to detoxify excess ROS (Agarwal, 2007; Agrawal & Rathore, 2007; Jain *et al.*, 2004; Landry *et al.*, 1995; Rao, Paliyath *et al.*, 1996; Ravindran *et al.*, 2008; Takeuchi *et al.*, 2007).

Increment of the protein content under UV might be due to the synthesis of stress proteins and other related enzymes. Similar trend of increment in protein content was reported in *Brassica napus* under UV-B stress (Nasibi, 2005).

CONCLUSION

The plants have several mechanisms against the deleterious effects of UV radiation which include a combination of damaging, repairing and tolerance, but this relevance is less known. Protective mechanisms against UV damage in plants is depends on plant species. The present study suggested that, *D. moldavica* is sensitive plant against high UV dosages. The increased activity of catalase, peroxidase and ascorbate peroxidase in high doses of UV treatment and the increased amount of anthocyanins and carotenoids in same doses shows the plant sensitivity levels in front of UV exposure and shows combination of enzymatic and non-enzymatic defence mechanisms, which decreased the effects of radiation damage in higher doses. Chlorophyll values and SOD decreasing in same doses with upper mentioned results of enzymes and pigments supported the mentioned mechanism of plant defence system against under 320 nm UV radiation. More studies should be done to understand the precise plant defence system against UV radiations.

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