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Combined Effects of Drought and UV Stress on Quantitative and Qualitative Properties of *Bunium persicum*

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Abstract: Research related to plant responses to combined stresses is very limited, especially in medicinal plants. Present investigation was conducted in order to evaluate the effects of drought and UV-B stress on physiological performance and chemical properties of major Iranian medicinal species of *Bunium persicum*. A factorial plan based on a randomized complete block design was performed with two irrigation levels [irrigation after 60 (control) and 150 mm (drought stress) evaporation from evaporation pan] and three UV-B treatments [no UV (control), 1 and 2 hr radiation] in three replications. Results showed that plants under normal water condition and 2 hr UV-B radiation produced a higher seed yield. Yield components were generally higher in normal water condition and 1 hr UV-B treatments, except for some parameters, including seed size. Plants which grown under UV radiation had higher content of essential oil (EO) compared with the ones that did not receive UV treatments. Contrary with the yield components, qualitative properties of EO were affected by the levels of applied treatments and drought stress, and the 2 hr UV-B treatments highly improved antioxidative properties of EO. 22-diphenyl 1-picrylhydrazyl radical (DPPH) scavenging activity, hydrogen peroxide scavenging activity, Fe reducing power and phenol content were enhanced by high UV-B radiation level and drought conditions. In general, some alleviative effects between drought and UV-B were observed in regards to yield and yield components of *B. persicum*, although they were not very considerable. The interaction of applied treatments was more obvious in antioxidative activities of essential oil.

Key words: Essential oil, Radiation, Scavenging activity, Yield.

Introduction

Environmental stresses are the most important reducing factors in agricultural productions around the world 1. Stresses tolerance induction is a complex process including various molecular, biochemical and physiological factors 2,3. It has also been known that under field conditions, plants are exposed to different abiotic stresses, simultaneously, which amplifies deleterious effects of stress conditions. Limited research related to plant responses to a combination of abiotic stresses brings the fact that this unique response cannot be easily interpreted from the response of plants to each individually applied stress. Despite these evidences, a study with focus on co-occurrence of stress condition is surprisingly neglected by researchers in this field 4. Ultraviolet-B (UV-B) radiation is an environmental factor affects cellular processes of plants. Stratospheric ozone layer has a key role in absorption of UV-B radiation. Anthropogenic activities in recent decades have led to the reduction
of stratospheric layer, and the UV-B radiation reaching the earth’s surface is consequently elevated. Unfortunately, this trend is predicted to increase until 2050.

In regards to the research related to UV-B effects on plants, it can be concluded that this radiation is generally known as a destructive factor for plant growth and productivity. On the other hand, some studies had shown that it could have some positive effects on some properties of plants. This side of UV-B effects is rarely beneficial for edible plants and is mostly related to medicinal plant species.

Bunium persicum is one of the most important medicinal plants of Iran, Pakistan, and the neighboring countries. Besides the long history of this plant as a valuable spice and culinary usage, several medicinal and antioxidative properties of B. persicum essential oil were found in the recent years. The content of the essential oil in this plant was reported up to 7.5% by the authors (unpublished data) and it could be used as a precious resource of secondary metabolites with antioxidant properties. Regrettably, the plant has been exposed into extinction danger in the recent years as a consequence of inappropriate and unscientific commercial collection of the seeds for rapid financial gains. Severe competition for the seed collection has led to removing the entire plant with undeveloped seeds.

Combined effects of environmental stresses have been synergistic or antagonistic. There are some reports related to the effects of drought and UV-B radiation on quantitative and qualitative properties of plants and the discussion is controversial. In fact, it was shown that drought could enhance or mask UV-B effects on plants. It is also considerable to note that majority of the research in this area is conducted under glasshouse conditions and only less than 5% of them are performed under field conditions.

Considering the importance of the studies on combined stresses and regarding the neglected sides of drought and UV-B effects on medicinal plants, the present investigation was conducted with the aim of evaluating the combination of drought and UV-B effects on B. persicum quantity and quality.

Materials and Methods

plant materials and growth conditions

The experiment was conducted at the Research Field of Ferdowsi University of Mashhad, Iran. In respect to the perennial nature of the plant, the study was performed on plant materials (tuberous roots) with the average age of 5 years. Tuberous roots were germinated in late winter (18 March) and turned into stem elongation about one month later (21 April). Drought treatments were applied via the irrigation intervals based on the evaporation from the evaporation pan at two levels, including 60 and 150. The UV treatments were applied using UV-B fluorescence lamps (280-315 nm). Four artificial chambers which covered by dark plastic with 100*125 cm dimensions were used and the UV-B lamps were hanged with approximately 100 cm distance above the plants. The UV-B treatments included control, 1, and 2 hours radiation every day. Treatments were applied after the beginning of flowering stage. The amount of water applied was measured using a volumetric water flow meter and was the same for all the similar treatments (150 liter for each plot for each time of irrigation). During the tuberous roots production and the experimental period (6 years) no herbicide, pesticide, chemical, or organic fertilizers were used. Plants were harvested after the physiological ripening (22 June 2012). Five plants from each plot were randomly sampled to determine the yield per plant and the yield components. The yield per area was also determined using a 1 m² quadrat. Seed samples from each plot (50 g) were used for essential oils extraction and biochemical analysis.

Essential oil extraction

Fifty (50) g of seed and 250 ml water were placed in a clevenger type apparatus. The essential oils were isolated by hydro distillation for 3 h. The obtained essential oil was stored in a sealed vial, at -20°C for further measurements.

Biochemical analysis

2'-diphenyl 1-picrylhydrazyl radical (DPPH) assay

Bleaching of the purple colored ethanol solution of DPPH was used to measure electron-do-
nation ability of B. Persicum essential oil. Two ml of various concentrations of the samples (0.045-0.45 % w/v) in ethanol were added to 1 ml of a 2×10^{-4} M solution of DPPH. The decrease in absorbance at 517 nm was determined by spectrophotometer after 30 min for all samples. Ethanol was used as blank. The absorbance of the ethanol solution DPPH radical without essential oil was measured as control. All the determinations were performed in triplicate and the results were averaged. DPPH radical inhibition by the sample percentage was calculated as follows:

\[
\text{% Inhibition} = \left( \frac{A_{c(0)} - A_{s(t)}}{A_{c(0)}} \right) \times 100
\]

where, \(A_{c(0)}\) is the absorbance of the control at \(t=0\) min and \(A_{s(t)}\) is the absorbance of the sample at \(t\). Essential oil concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotting percentage of remaining DPPH against essential oil concentration.

**Hydrogen Peroxide Scavenging Activity**

The ability of the essential oil to scavenge hydrogen peroxide was determined according to the method described by Nabavi et al. A hydrogen peroxide solution (40 mM) was provided in phosphate buffer (pH 7.4). The concentration of hydrogen peroxide was determined by spectrophotometer absorption at 230 nm. Extracts (0.1-1 mg ml) in distilled water were added to 0.6 ml hydrogen peroxide solution (40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Essential oil and hydrogen peroxide scavenging percentage were calculated by the following formula.

\[
\text{% scavenging} = \left( \frac{A_o - A_t}{A_o} \right) \times 100
\]

where, \(A_o\) is the absorbance of the control and \(A_t\) is the absorbance in the presence of the essential oil and standard compounds.

**Phenolic Compounds**

Total phenolic compound contents were determined according to the Folin-Ciocalteu method. The extract samples (0.5 ml) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na2CO3 (4 ml, 1 M) were then added. The mixture was stored for 15 min and the phenols were determined according to the absorption at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg.ml solutions of gallic acid in ethanol: water (50:50, v.v). Values are expressed in terms of gallic acid equivalent (mg.100g dry mass).

**Fe-reducing Power Assay**

Fe-reducing power was determined according to the method described by Hsu et al. One ml of various concentrations of sample was mixed with potassium ferricyanide (500 μl, 1% w.w in water) and 500 μl of 0.2 M phosphate buffer. The solutions were stored in water bath at 50°C for 20 min. Then, 500 μl of trichloroacetic acid (10 % w.w) was added and centrifuged for 10 min at 3,000 rpm. Afterwards, 500 μl of transparent supernatant was separated and added to 100 μl of ferric chloride. The solution absorption was recorded after 30 min at 700 nm. The increase in absorption of reaction mixture indicates the increase in reducing power of essential oils.

**Essential Oil Chemical Composition Determination**

Chemical constituents of EO samples were recognized by GC-MS analyses. The GC-MS apparatus was a Varian GC-MS spectrometer consisting of a Varian Star 3400 GC equipped with a fused-silica column (DB-5, 30 m x 0.25 mm i.d., film thickness 0.25 μm; J and W Scientific Inc.), interfaced with a mass spectrometric detector (Varian Saturn 3). The components of essential oil were identified by using their retention indices (RI) obtained with reference to the n-alkane series (Sigma, UK) on the DB-5 column, mass spectra with those of authentic samples, composition of their mass spectra and fragmentation patterns reported in the literature, and computer matching with MS-data bank (Saturn version 4). Quantification of the relative amount of the individual components was performed according to the area percentage method.
Statistical analysis

A factorial plan based on a randomized complete block design with six treatments and three replications were conducted. All data were analyzed using Analysis of Variance (ANOVA) and the LSD was calculated at P = 0.05 for the means comparisons.

Results and discussion

Yield and yield components

The UV radiation treatments affected plant’s yield under different irrigation levels. When plants were exposed to 1 hr UV treatment, yield was reduced under both irrigation levels. However, with increasing UV treatment period to 2 hr, seed yields were increased, but they were lower than control (no UV-B treatments). Results of yield per area also showed the same trend.

Umbel number per plant was not significantly affected by the applied treatments. The highest number of umbel was achieved under control and the lowest was observed when plants were under drought conditions and 2 hr UV radiation. Umbellet number per umbel was not influenced significantly by water deficit and UV treatments and the highest umbellet number was observed on plants under normal water conditions and 1 hr UV treatment. Consequently, umbellet number per plant did not show any significant difference between the treatments.

Decreasing yield and yield components of B. persicum in response to drought conditions was expectable and similar to the results of previous study of authors about the effects of drought on yield and yield components of B. persicum (unpublished data). The UV-B reducing effect was also obvious among the treatments, even though the difference between different levels of UV-B was not significant in regards to the yield. This implies that drought and UV-B treatments may have alleviative effects on each other.

Increasing branch numbers was reported as a common consequence of plants exposure to the UV-B radiation, especially in the low plant intensity. This response is mainly considered as plant morphogenetic responses under UV-B stress 18.

The highest number of seed per umbellet was obtained in plants under normal conditions. The UV-B treatment decreased seed number on both water treatments and when plants were grown under drought conditions, the 2 hr UV treatment produced the lowest seed number. Considering the results of the two mentioned index, seed number per plant showed a similar trend to a great extent. Plants under the 2 hr UV treatment and normal and water stress conditions had the highest and the lowest values of seed size, respectively. It is necessary to consider that seed size trend is highly comparable with yield trend, especially with yield per area. The highest yield per area was obtained under normal water condition and 2 hr UV treatment (Fig 1.B). Highest seed size value was also achieved in the same treatment (Fig 2.D). Considering the trend of other yield components, this similarity is seems to be important.

Plant height did not show significant difference between the treatments, although plants under control conditions had the highest height values and plants grown under drought conditions and treated by UV radiation had higher height compared with plants treated by UV under normal water conditions. Reducing height value is a result of UV treatment and a physiological response due to alteration of cell division rate and duration. Indole acetic acid (IAA) is a chemical compound which assumed to have a key role in this process 19. Even though decreasing plant height due to UV-B treatment was not considerable in this experiment, reduced height was previously reported in Solanum tuberosum 20 and Spirodela polyrhiza 21 treated by UV-B radiation.

Essential oil percentage and seed yield

Essential oil (EO) content of seeds was influenced by the applied treatments. Plants grown under UV radiation had higher content of EO compared with plants which not received UV treatments. The highest percentage of EO was achieved in plants under 2 hr UV treatment and normal irrigation. EO yield which depends on EO percentage and seed yield showed significant difference between the treatments and plants under normal water conditions and the 2 hr UV radiation had the highest EO yield. Higher EO yield of plants under normal water condition and non treated with UV radiation is mainly related to high
Figure 1. *B. persicum* seed yield per plant (A) and area (B), umbel (C) and umbellet (D) number per plant under normal (irrigation after 60 mm evaporation from evaporation pan) and drought (150 mm evaporation from evaporation pan) conditions and different levels of UV-B treatments (control, 1 and 2 hr radiation every day). Data are the mean values of three replications ±SE which represented by the vertical bar in each graph.

seed yield in these treatments. Essential oil content is one of the most important properties which has been affected by UV radiation in medicinal plants. There are some reports indicate that UV-B radiation could enhance essential oil production in medicinal plants, including *Mentha spicata* and *Ocimum basilicum*. According to these results, enhancing effects of UV-B on EO content of *B. persicum* is obvious, even though the combination of UV and drought conditions did not show a synergistic effect on EO content.

Antioxidative activity

UV-B radiation could induce reactive oxygen species (ROS) production which leads to oxidative damage and plants evolved antioxidative defense system to protect cell structures. ROS can also be generated via excitation of UV-B receptors activating nitrogenmonoxide (NO) signaling and NADPH oxidase. The results of the present experiment indicated that UV-B treatments along with drought condition or normal water condition could amplify antioxidative response of *B.*
persicum through the enhancing scavenging activity of EO.

DPPH assay

The highest DPPH scavenging activity (the lowest IC₅₀) was recorded on plants under drought condition that received 2 hr UV radiation. Plants under the same water availability and 1 hr UV treatment had also high scavenging activity and showed significant difference with other treatments. The lowest scavenging activity belonged to plants under control conditions. So, increased scavenging activity of B. persicum EO could be considered as a result of the UV-B treatment which was also enhanced due to the synergistic effect of drought condition. Enhancing role of drought on DPPH scavenging activity has been reported by some researchers. For instance, Zhu
et al., showed intensifying effect of drought condition on DPPH scavenging activity of *Bupleurum* spp. In another study, effects of drought on the seeds extract antioxidants activity of *Cuminum cyminum* by different methods were investigated and it was shown that seeds under drought conditions had a higher antioxidants activity.

**Hydrogen peroxide scavenging activity**

Results revealed that applied treatments significantly affected hydrogen peroxide scavenging activity. Similar to the DPPH assessment results, the highest scavenging activity was observed on plants under drought condition with 2 hr UV radiation. The considerable point was that the plants grown under drought that were not treated with UV did not have significant difference regarding the highest values of scavenging activity. It seems that hydrogen peroxide scavenging activity was more affected by drought conditions than UV treatment. The importance of the hydrogen per-
Figure 4. Antioxidative properties of B. persicum plants, including DPPH scavenging activity (IC50 value) (A), Hydrogen proxide scavenging activity (inhibition%) (B), Phenol content (mg/ml) (C) and Fe-reducing power (absorption) (D) under normal (60 mm evaporation from evaporation basin) and drought (150 mm evaporation from evaporation basin) conditions and different levels of UV-B treatments (control, 1, and 2 hr radiation every day). Data are the mean values of three replications ±SE which represented by the vertical bar in each graph.

Oxide scavenging activity in protecting cellular structures was previously assessed and documented.

**Phenol content**

The higher values of phenol content were found in plants treated by UV radiation. Along with increasing UV treatment time, phenol content was also elevated. Plants which were not treated by UV and were under drought condition had also high amounts of phenol content. Increasing in phenolic compounds is a physiological response of plants under UV-B radiation and it was shown that this process is a protective mechanism through scavenging nature of phenolic compounds. Besides the effect of UV-B in enhancing phenolic compounds accumulation, the role of drought in increasing phenolic compound in
this experiment was also considerable.

Fe reducing power
Drought and UV treatments had a considerable effect on Fe reducing power of plants and the highest values of reducing power were observed in plants grown under drought and UV radiation, even though the difference between plants under 1 and 2 hr UV treatments and plants which did not receive UV radiation was not significant. So, it can be concluded that EO reducing power is an index with more dependence on water availability and UV treatment is not a determined factor in reducing power. Likewise, it was shown that reducing power of C. cyminum extract was higher when plants were encountered different levels of drought during the growth period.

Chemical compositions of essential oil
In essential oil analysis, 25 compounds were detected which 22 compounds were recognized in all samples. γ-terpinene had the highest percentage (37.3-43.4) and γ-terpinen-7-al (13.1-14.9) and p-cuminaldehyde (13.4-15.3) had higher percentages compared with other compounds. 1.8-cineole (5-5.3), p-cymene (4.3-4.6) and limonene

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were the other chemical compounds with high relative percentage. According to the achieved results, combined treatments of drought and UV-B did not show any significant effect on chemical composition of the essential oil of *B. persicum*, although some differences were observed which were not considerable.

Chemical composition of essential oil of *B. persicum* was studied by some researchers. Azizi *et al.* analyzed EO constituents of *B. persicum* under wild and agronomical conditions and no significant difference was observed. In general, EO constituent is a character which mainly does not affected by environmental stresses, significantly.

Based on the results of the present study, it can be concluded that drought and UV-B had alleviative interactions in regards to some of the quantitative properties and showed synergistic effects in terms of qualitative properties and EO antioxidative activities of *B. persicum*. Further investigation about the longer periods of drought and UV-B effects is recommended.

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