

Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*

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Abstract: Drought stress is one of the growing concerns in agriculture management around the world. Adopting tolerant plants to drought conditions could be an appropriate approach to this problem. *Bunium persicum* is one of the most important medicinal plants of Iran and Turkey as well as neighbouring countries and has been forced into endangered plant status due to mismanagement of its wild habitats. An experiment was conducted in order to evaluate drought tolerance of *Bunium persicum* and the effects of drought on its essential oil qualitative properties. Two separate plans based on randomised complete block design were performed with 4 drought levels (irrigation after 60, 90, 120, and 150 mm of evaporation from an evaporation basin) and 3 replications. Drought treatments were applied after the stem elongation and flowering stages. Results indicated that applied drought treatments reduced yield and yield components of plants in both growth stages. Some differences were observed in yield components when plants were exposed to drought stress at different growth stages. The essential oil percentage of *Bunium persicum* was affected by drought conditions, and it was elevated along with increasing drought levels. Due to reduced seed yield, essential oil yield was significantly decreased. Antioxidative activity assessment of seed extracts revealed that drought had positive effects on antioxidant parameters, including 2'-diphenyl 1-picrylhydrazyl radical scavenging activity, hydrogen peroxide scavenging activity, and Fe-reducing power. Phenol content was also improved through applied drought treatments. In general, these results showed the high tolerance of *Bunium persicum* to drought and also revealed positive effects of drought on the antioxidative activities of plant seeds.

Key words: Drought stress, medicinal plants, reducing power

1. Introduction

Due to the growth of population and expansion of the agricultural, energy, and industrial sectors, the demand for water has increased extensively, and water scarcity has been occurring almost every year in many parts of the world (Mishra and Singh, 2010). Drought is known as a major abiotic factor that limits plant's growth and production. Although the general effects of drought on plant growth are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well understood (Bhatnagar-Mathur et al., 2009). Furthermore, the physiologic and metabolic responses of crops to dry environments have been well studied, but similar studies are lacking in medicinal and aromatic plants.

Plants subjected to environmental stress evolved a complex and efficient antioxidant system, which includes enzymatic antioxidants and nonenzymatic antioxidants to counteract the detrimental effects of active oxygen species (Zhu et al., 2009). These are toxic intermediates that result from a reduction in molecular O₂, including superoxide

anion (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH) (Dat et al., 2000).

The role of antioxidative defence systems in plant responses to drought stress was comprehensively documented in *Gypsophila aucheri*, which is a xerophytic plant (Sekmen Esen et al., 2012). In another study, antioxidative and physiological responses of 2 sunflower (*Helianthus annuus*) cultivars under drought stress were evaluated, and the efficiency of antioxidative systems in coping with drought effects was clear (Baloğlu et al., 2012).

It was also shown that a plant's ability to cope with abiotic stress is mainly related to an altered biochemical profile and produces a varied range of secondary metabolites. Secondary metabolite production is a critical part of the defence response to stress conditions. The role of lipid peroxidation in initiation of secondary metabolites has been documented by some researchers. Consequently, the accumulation of secondary metabolites is mainly related to membrane lipid protection from oxidative stress, and reactive oxygen species (ROS) are the mediators in the

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biosynthesis of particular secondary metabolites (Zhu et al., 2009).

Water stress decreases growth of some medicinal plants, including *Hypericum brasiliense* Choisy (Nacif de Abreu and Mazzafera, 2005) and *Bupleurum chinense* DC. (Zhu et al., 2009). On the other hand, many studies have shown that drought enhances the amount of secondary metabolites in a wide variety of plant species, such as *Rehmannia glutinosa* (Gaertn.) DC. (Chung et al., 2006). Conversely, drought caused a significant reduction in all growth parameters and essential oil yield and percentage in some medicinal plants such as peppermint (*Mentha piperita* L.) (Khorasaninejad et al., 2011).

Bunium persicum (Boiss.) is a medicinal plant belonging to the family Apiaceae and it grows in different regions of Iran. Its seeds are called “zireh kuhi” and “zira kermani” and are used as a culinary spice (Mortazavi et al., 2010). In the indigenous system of medicines, these seeds are regarded as stimulants and carminatives and are useful for treating diarrhoea and dyspepsia. The plant has been forced into the category of endangered plants due to the recent and relentless extraction of seeds from wild habitats. The main depletion factor and cause of endangerment of this species has been the thoughtless, improper, and unscientific commercial collection of seeds for rapid financial gain. The competition for its seeds is so severe that, instead of collecting the ripe seed, the entire plant is removed, even when the seeds are immature (Azizi et al., 2009). If this irresponsible trend in harvesting the seeds of this plant is continued, it will lead to the extinction of this valuable and endangered species.

To date, there is no published and comprehensive research related to drought impacts on *Bunium persicum* characteristics. Therefore, the present investigation was conducted with the aim of evaluating drought impacts on quantitative and qualitative properties of *Bunium persicum*.

2. Materials and methods

2.1. Plant growth conditions

The experiment was conducted at the research field of Ferdowsi University of Mashhad, Iran. In regard to the perennial nature of plant, the study was performed on plant materials (tuberous roots) with an average age of 5 years. Tuberous roots were germinated in late winter (18 March) and reached stem elongation about 1 month later (21 April). Two separate plans based on randomised complete block design with 3 replications were carried out to evaluate drought impact at 2 growth stages, stem elongation and flowering. Drought treatments were applied through the irrigation period based on the evaporation from the evaporation basin at 4 levels: 60, 90, 120, and 150 mm. In the first plan, drought treatments

were applied at the beginning of the stem elongation stage until the physiological ripening, and in the second plan, drought treatments were applied at the flowering stage (when about 50% of plants started flowering). The amount of water input was measured using a water meter and was the same for all similar treatments. During tuberous root production and the experimental period (6 years) no herbicides, pesticides, chemicals, or organic fertilisers were used. Plants were harvested after physiological ripening (20 June 2012). Five plants from each plot were randomly sampled to determine the yield per plant and 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 oil extraction and biochemical analysis.

2.2. Essential oil isolation

Fifty grams of seeds and 250 mL of water were placed in a Clevenger-type apparatus. The essential oil was isolated by hydrodistillation for 3 h (Bernath, 1990). The obtained essential oil was stored in a sealed vial at -20 °C for further measurements.

2.3. Biochemical analysis

2.3.1. 2'-Diphenyl 1-picrylhydrazyl radical assay

Bleaching of the purple-coloured ethanol solution of 2'-diphenyl 1-picrylhydrazyl radical (DPPH) was used to measure the electron-donating ability of *Bunium persicum* essential oil (Yamaguchi et al., 1998), and DPPH was employed as a reagent. Two millilitres 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 a blank. The absorbance of the ethanol solution DPPH radical without essential oil was measured as the control. All the determinations were performed in triplicate, and the results were averaged. DPPH radical inhibition by the sample percentage was calculated as follows: % inhibition = $[(A_{c(0)} - A_{s(t)}) / A_{c(0)}] \times 100$, 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.

2.3.2. 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. (2008). 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⁻¹) in distilled water were added to 0.6 mL of hydrogen peroxide solution (40

Table 1. Essential oil chemical compositions of *Bunium persicum* plants under different levels of drought stress applied after stem elongation stage (values are given as percentages).

| Compound name | Treatments | | | |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| | DT ₁ | DT ₂ | DT ₃ | DT ₄ |
| Borneol | 3.0 | 3.0 | 3.0 | 2.0 |
| Isosylvestrene | 4.0 | 2.0 | 2.0 | 3.0 |
| cis-Sabinene hydrate | 2.0 | 2.0 | 2.0 | 2.0 |
| Sabinene | 7.0 | 9.0 | 6.0 | 6.0 |
| Camphene | 3.0 | 3.0 | 2.0 | 3.0 |
| α -Pinene | 7.1 | 5.1 | 8.1 | 7.1 |
| Myrcene | 3.1 | 4.1 | 4.1 | 4.1 |
| 1.8-Cineole | 4.3 | 2.4 | 7.3 | 9.3 |
| Linalool | 4.0 | 3.0 | 3.0 | - |
| Terpinen-4-ol | 2.0 | 2.0 | 3.0 | 2.0 |
| α -Terpineol | 1.0 | 1.0 | 1.0 | 2.0 |
| Perillaldehyde | 3.0 | 3.0 | 3.0 | 2.0 |
| α -Terpinen-7-al | 2.0 | 3.0 | 3.0 | 3.0 |
| γ -Terpinen-7-al | 3.15 | 16 | 7.15 | 9.13 |
| Germacrene D | 1.0 | 1.0 | 1.0 | 1.0 |
| α -Thujene | 2.0 | 2.0 | 3.0 | 2.0 |
| β -Pinene | 3.1 | 1.1 | 7.1 | 7.1 |
| 3-Methylbenzaldehyde | 1.0 | 1.0 | - | 1.0 |
| β -Pinene | 3.1 | 1.1 | 7.1 | 7.1 |
| Limonene | 3 | 3.3 | 2.3 | 3.3 |
| Terpinolene | 4.0 | 6.0 | 4.0 | 4.0 |
| γ -Terpinene | 5.41 | 3.40 | 1.42 | 5.42 |
| p-Cuminaldehyde | 7.14 | 2.14 | 9.14 | 1.41 |
| Bornyl acetate | 4.2 | 2.2 | 4.2 | 3.2 |
| Thymol | 1.0 | 1.0 | - | 1.0 |
| ar-Curcumene | 2.0 | 2.0 | 1.0 | 2.0 |
| Zingiberene | 1.0 | 2.0 | 1.0 | 2.0 |
| β -Sesquiphellandrene | 2.0 | 2.0 | 1.0 | 1.0 |
| p-Cymene | 4.5 | 2.5 | 5.4 | 5.5 |

Table 2. Essential oil chemical composition of *Bunium persicum* plants under different levels of drought stress applied after flowering stage (values are given as percentages).

| Compound name | Treatments | | | |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|
| | DT ₁ | DT ₂ | DT ₃ | DT ₄ |
| Isosylvestrene | 3.0 | 2.0 | 3.0 | 3.0 |
| cis-Sabinene hydrate | 4.0 | 3.0 | 3.0 | 4.0 |
| Sabinene | 5.0 | 6.0 | 5.0 | 7.0 |
| Camphene | 4.0 | 3.0 | 3.0 | 3.0 |
| α -Pinene | 4.1 | 2.1 | 7.1 | 4.1 |
| Myrcene | 5.1 | 7.1 | 4.1 | 3.1 |
| 1.8-Cineole | 1.4 | 9.3 | 7.3 | 9.3 |
| Linalool | 3.0 | 4.0 | 2.0 | 2.0 |
| Terpinen-4-ol | 4.0 | 3.0 | 3.0 | 2.0 |
| α -Terpineol | 2.0 | 1.0 | - | 2.0 |
| Perillaldehyde | 5.0 | 3.0 | 2.0 | 2.0 |
| α -Terpinen-7-al | 3.0 | 3.0 | 1.0 | 3.0 |
| γ -Terpinen-7-al | 2.13 | 7.13 | 1.16 | 16 |
| Germacrene D | 2.0 | 1.0 | 2.0 | 2.0 |
| α -Thujene | 4.0 | 2.0 | 2.0 | 3.0 |
| β -Pinene | 9.1 | 9.1 | 5.1 | 7.1 |
| 3-Methylbenzaldehyde | | | | |
| Limonene | 7.2 | 7.2 | 3.2 | 7.2 |
| Terpinolene | 5.0 | 3.0 | 6.0 | 7.0 |
| γ -Terpinene | 2.39 | 1.43 | 7.41 | 1.41 |
| p-Cuminaldehyde | 5.14 | 14 | 3.15 | 1.15 |
| Bornyl acetate | 4.3 | 1.3 | 2.2 | 5.2 |
| Thymol | 1.0 | - | 1.0 | 1.0 |
| ar-Curcumene | 1.0 | 1.0 | - | 1.0 |
| β -Sesquiphellandrene | 1.0 | 2.0 | - | 1.0 |
| p-Cymene | 1.6 | 4.6 | 1.6 | 1.6 |
| trans-Sabinene hydrate | 1.0 | 1.0 | - | 1.0 |

mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Essential oil and hydrogen peroxide scavenging percentage was calculated by the following formulas: % scavenging = $[(A_0 - A_1) / A_0] \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oil and standard compounds.

2.3.3. Phenolic compounds

Total phenolic compound contents were determined according to the Folin–Ciocalteu method (Waterhouse, 2002). 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 Na_2CO_3 (4 mL, 1 M) was 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 with 0, 50, 100, 150, 200, and 250 mg mL^{-1} solutions of gallic acid in ethanol and water (50:50, v/v). Values are expressed in terms of gallic acid equivalent ($\text{mg } 100 \text{ g}^{-1}$ dry mass).

2.3.4. Fe-reducing power assay

Fe-reducing power was determined according to the method described by Hsu et al. (2006). One millilitre 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 a water bath at 50 °C for 20 min. Next, 500 μL of trichloroacetic acid (10% w/w) was added and centrifuged for 10 min at 3000 rpm. After that, 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 the absorption of the reaction mixture indicates the increase in the reducing power of essential oils.

2.4. Essential oil chemical composition determination

Chemical constituents of essential oil samples were recognised by gas chromatography–mass spectrometry (GC–MS) analyses. The GC–MS apparatus was a Varian GC-MS spectrometer consisting of a Varian Star 3400 gas chromatograph equipped with a fused-silica column (DB-5, 30 m \times 0.25 mm i.d., film thickness 0.25 μm ; J and W Scientific, Inc.), interfaced with a mass spectrometric detector (Varian Saturn 3). Essential oil components were identified by using retention indices obtained with reference to the n-alkane series (Sigma, UK) on the DB-5 column, mass spectra using authentic samples, composition of mass spectra and fragmentation patterns from the literature, and computer matching with a MS-data bank (Saturn, version 4) (Adams, 2001). Quantification of the relative amount of individual components was performed according to the area percentage method.

3. Results and discussion

3.1. Yield

Bunium persicum yield was affected under drought conditions in both growth stages (Figure 1). The highest yield per plant and per area was obtained at the second level of the drought treatments (DT_2), and it declined along with increasing drought levels. Comparing the results of yield per plant, it is obvious that when plants encountered drought at the flowering stage, yield was similar to that of plants under drought conditions from the stem elongation stage to a great extent. The yield per area also showed a similar trend. Reduced yield of *Bunium persicum* in response to drought conditions implied a plant tolerance level to drought conditions. In fact, the second level of drought not only failed to decrease yield, but also enhanced it to a greater level than the first treatment.

Enhanced yield at the second level of drought treatment compared with the first revealed that this level of irrigation may be more appropriate and could produce higher water-use efficiency for *Bunium persicum*. Drought impacts on crop and medicinal plant yields have been reported by some researchers (Mohamed and Abdu, 2004; Renau-Morata et al., 2012).

3.2. Yield components

The components of *Bunium persicum* yield were also affected by drought conditions. The number of umbels per plant was reduced due to applied drought treatments at both growth stages (Figure 1). Even though it was slightly decreased at the second level of drought treatment, the differences were not significant. Drought conditions reduced the umbellet number per umbel, but plants under drought conditions from the stem elongation stage showed no significant differences among the 3 levels of drought treatment (Figure 1). This may imply that plants had an adaptation strategy to drought conditions during the growing season. The number of umbellets in the drought application from stem elongation was lower compared with plants under drought conditions from the reproductive stage, which revealed that water availability could enhance umbellet production depending on the growth stage of drought application (Figure 2). Number of seeds per umbellet is also affected by drought treatments (Figure 2). Plants under drought conditions from the reproductive stage did not show significant differences among 3 levels of drought treatment. In general, seed numbers per umbellet were statistically similar in both growth stages. Seed formation in umbellets is a critical factor for determining the final yield of plants belonging to the family Apiaceae. Hence, any factor that affects this parameter will affect the final yield. Seed number per plant is a yield component that is determined by other components and could be considered an index showing how applied treatments affect yield components. Seeds

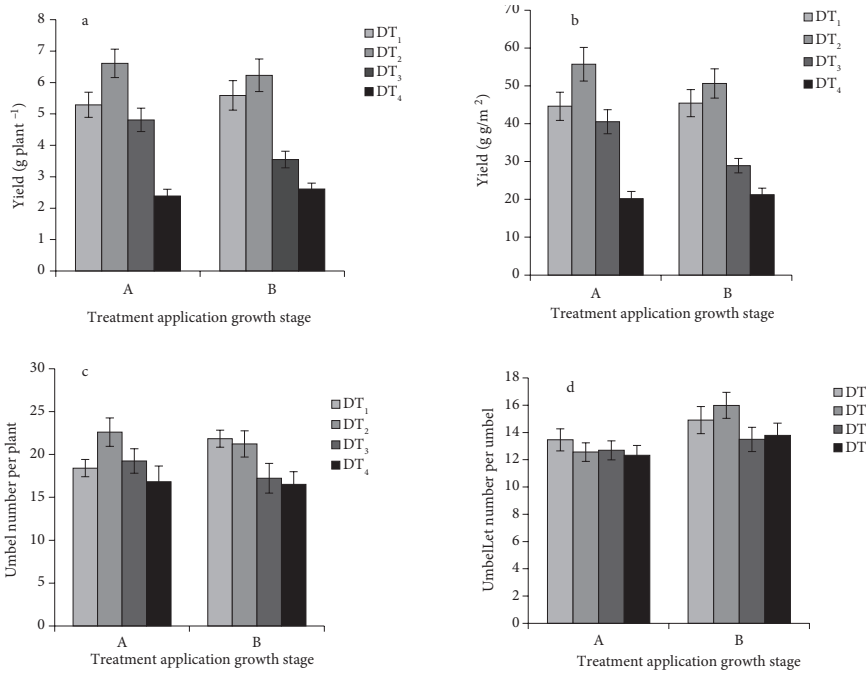


Figure 1. Yield per plant (a) and area (b), and umbel (c) and umbellet (d) number per plant of *Bunium persicum* plants under different drought levels (DT₁ = 60, DT₂ = 90, DT₃ = 120, and DT₄ = 150 mm evaporation from evaporation basin) and growth stages (A = stem elongation stage and B = reproductive stage). Data are the mean values of 3 replications ± SE, represented by the vertical bar in each graph.

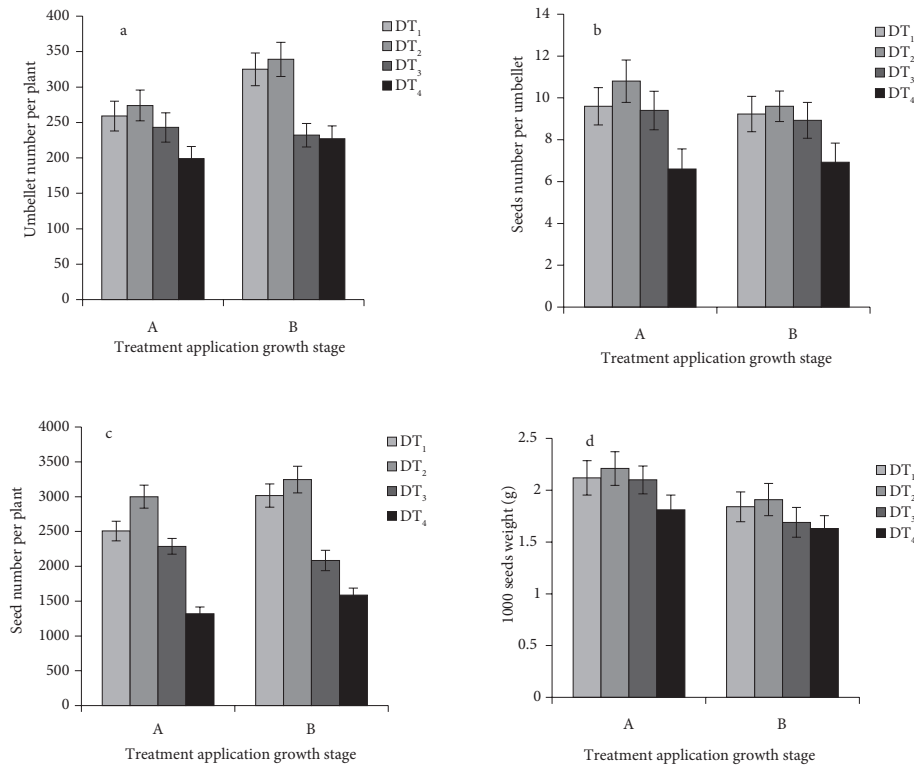


Figure 2. Umbel number per plant (a), seed number per umbellet (b), seed number per plant (c), and 1000-seed weight (g) (d) of *Bunium persicum* plants under different drought levels (DT₁ = 60, DT₂ = 90, DT₃ = 120, and DT₄ = 150 mm evaporation from evaporation basin) and growth stages (A = stem elongation stage and B = reproductive stage). Data are the mean values of 3 replications ± SE, represented by the vertical bar in each graph.

per plant were substantially reduced as a result of severe drought (Figure 2). Seed size was affected by drought treatments (Figure 2). In addition to the reducing effect of drought on seed weight, it is notable that plants with lower umbel and umbellet numbers showed higher seed weights. This could be a result of greater allocation of dry matter produced to seed weight, compensating for the lower yield components of plants under severe drought stress.

Considering the results of plant height, significant differences were observed among treatments, although trends in the 2 plans were not similar (Figure 3). In fact, plants under drought conditions at the stem elongation stage were affected more severely than at the flowering stage, and the height decreased along with increasing drought levels. Considering that plant height is mainly dependent on source availability (water in particular) in the vegetative stage and that *Bunium persicum* is not an agronomic and domesticated plant showing particular morphological properties, these results seem reasonable. Drought stress effects on morphological properties and yield components of chamomile were also investigated, and similar reductions in the above-mentioned properties were reported (Baczek-Kwinta et al., 2009). The biological yield of plants exposed to drought at both growth stages also revealed a decreasing trend in general. Leaf and stem biomass production also showed a similar trend (Figure 3). Biomass production is usually considered a drought tolerance criterion, and these findings are similar to other study results to a great extent (Purcell et al., 2000).

3.3. Essential oil content

Essential oil percentage was affected by applied treatments (Figure 3). Drought generally increased essential oil content, especially in plants under the 120- and 150-mm treatments. The highest percentage of essential oil content was obtained with 150-mm treatments (7.67% and 7.34%). It should be noted that plants under drought conditions from the stem elongation stage showed a higher essential oil percentage. These results imply that longer drought periods could lead to an elevated essential oil content in *Bunium persicum* seeds. Although plants under drought treatment from the reproductive stage showed higher essential oil content along with increasing drought severity, the difference between them was not generally significant. Considering the fact that essential oil is accumulated after the reproductive stage, drought could have had a more serious effect on the essential oil content. According to the results of the present study, *Bunium persicum* may have a threshold related to drought effects on essential oil content, and higher levels could not significantly enhance essential oil accumulation. Enhanced essential oil content as a result of drought conditions was previously documented for some medicinal plants such as parsley [*Petroselinum crispum* (Mill.)] (Petropoulos et al., 2008) and *Satureja*

hortensis L. (Baher et al., 2002).

Essential oil yield is a dependent variable determined by seed yield and essential oil percentage. The highest essential oil yield (3.63 g m⁻²) was achieved on DT₂ treatments in plants exposed to drought after the reproductive stage. Significant differences were also observed among all treatments (Figure 3).

3.4. Antioxidative activity

3.4.1. DPPH method

Using the DPPH method, results demonstrated that through applied treatments, antioxidant activity was enhanced in plants under drought at both growth stages (Figure 4). The lowest IC₅₀ levels (0.71 and 0.74) were obtained in DT₄ treatments in both experiments. The IC₅₀ of DPPH activity is known as a valid index to determine the antioxidant activity of chemical compounds such as essential oils. A high IC₅₀ value in a sample reveals low antioxidant activity. Hence, it could be argued that drought intensified the antioxidant activity of *Bunium persicum* seeds based on the results of the DPPH assay. This result also showed that the amount of DPPH activity in this plant depends on the severity of the applied drought.

DPPH has been widely used for determination of the antioxidant activity of single compounds as well as of different plant extracts. The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen-donating antioxidant (Jung et al., 2008). There are some limited studies about the effects of drought on the antioxidant activity of medicinal plants. For instance, Zhu et al. (2009) found that drought conditions could intensify DPPH scavenging activity of *Bupleurum* spp. In another study, the effects of drought on the seed extract antioxidant activity of *Cuminum cyminum* L. by different methods were investigated, and it was shown that seeds under drought conditions had higher antioxidant activity (Rebey et al., 2012). *Cuminum cyminum* and *Bunium persicum* belong to the same family and have similar physiological properties.

3.4.2. Hydrogen peroxide scavenging

The applied treatments affected hydrogen peroxide scavenging activity of *Bunium persicum* (Figure 4). In general, IC₅₀ values declined as the level of drought increased. This trend at both growth stages was similar to a great extent. Hydrogen peroxide plays a critical role in biological functions, and it is known as a potentially toxic compound due to its ability to penetrate the cell membrane (Gulcin, 2007). It also produces highly activated hydroxyl radicals, which could harm cellular structures (Wettasinghe and Shahidi, 1999). Based on the results of the present study, drought applied at both growth stages amplified the antioxidant activity of *Bunium persicum*, and it was enhanced with increasing drought levels.

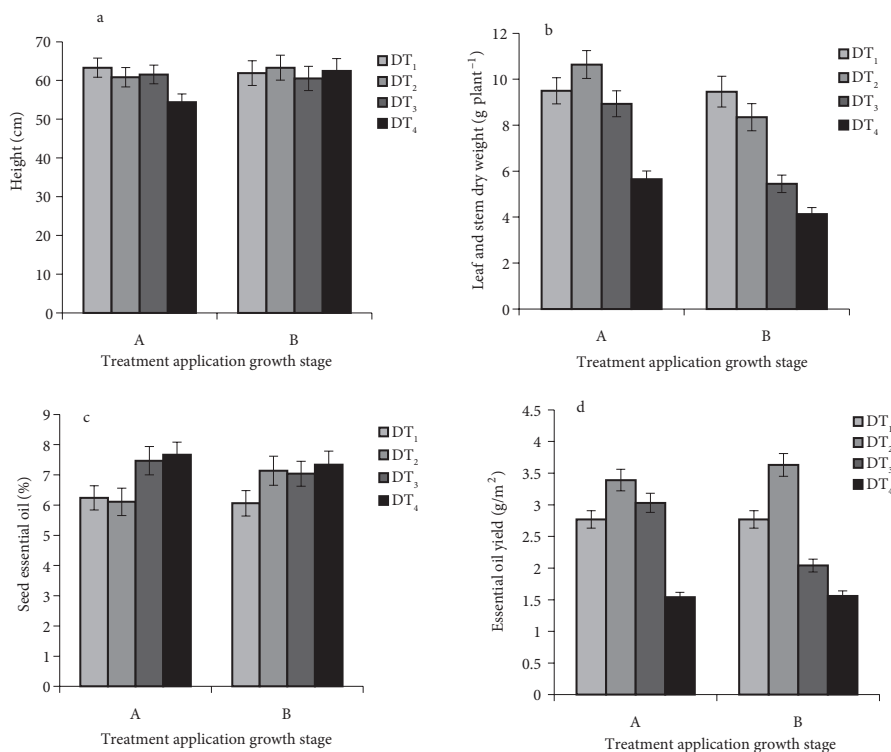


Figure 3. Height (cm) (a), leaf and stem dry weight (g plant⁻¹) (b), seed essential oil (%) (c), and essential oil yield (g/m²) (d) of *Bunium persicum* plants under different drought levels (DT₁ = 60, DT₂ = 90, DT₃ = 120, and DT₄ = 150 mm evaporation from evaporation basin) and growth stages (A = stem elongation stage and B = reproductive stage). Data are the mean values of 3 replications ± SE, represented by the vertical bar in each graph.

3.4.3. Fe-reducing power

The results of the Fe-reducing power assessment revealed higher reducing potential of *Bunium persicum* in response to drought conditions (Figure 4). The intensifying effect of drought on Fe-reducing potential was obvious in the evaluated treatments. The growth stage of the treatment application did not show significant effects on the Fe-reducing power of *B. persicum*. Fe-reducing power is another sign of antioxidative properties. Higher concentrations of reducing compounds usually lead to enhanced reduction of complex Fe³⁺/ferricyanide to ferrous. These findings imply that *Bunium persicum* essential oil is a rich source of reducing agents, which could play a key role in essential oil antioxidant activity. It is also clear that the amounts of these compounds were elevated with applied drought treatments. The reducing power of *Cuminum cyminum* extract was shown to be higher when plants encountered different levels of drought during their growth period (Rebey et al., 2012). According to Huang et al. (2006), this reducing power is essentially related to phenolic compounds of seed extracts. This theory was evaluated and supported by other studies in *Coriandrum sativum* (Neffati et al., 2010) and *Cakile maritima* Scop. (Ksouri et al., 2007).

3.4.4. Phenol content

Phenol concentration was improved when plants were exposed to drought conditions at both stem elongation and flowering stages (Figure 4). The phenol contents of plants under similar drought levels, but at different growth stages, were parallel, and no difference was observed between them. Phenol concentration was substantially increased in DT₄, the most severe drought treatment. This result demonstrated that phenol content of *Bunium persicum* essential oil is a drought-dependent variable, especially at higher levels of drought. The production of ROS that cause damage to the cellular apparatus is a common consequence of drought at the cellular level (Terzi et al., 2010; Makbul et al., 2011). The main importance of phenol compounds is related to the inhibitory potential of their hydroxyl group for free radicals. Hence, an elevated level of this compound could be considered an index of enhanced antioxidative activity of essential oil. Elevated phenolic content as a consequence of drought conditions was previously shown by some researchers (Zhu et al., 2009).

3.5. Chemical constituents of essential oil

In samples of plants under drought stress from the stem elongation stage, 28 compounds were detected, and 25

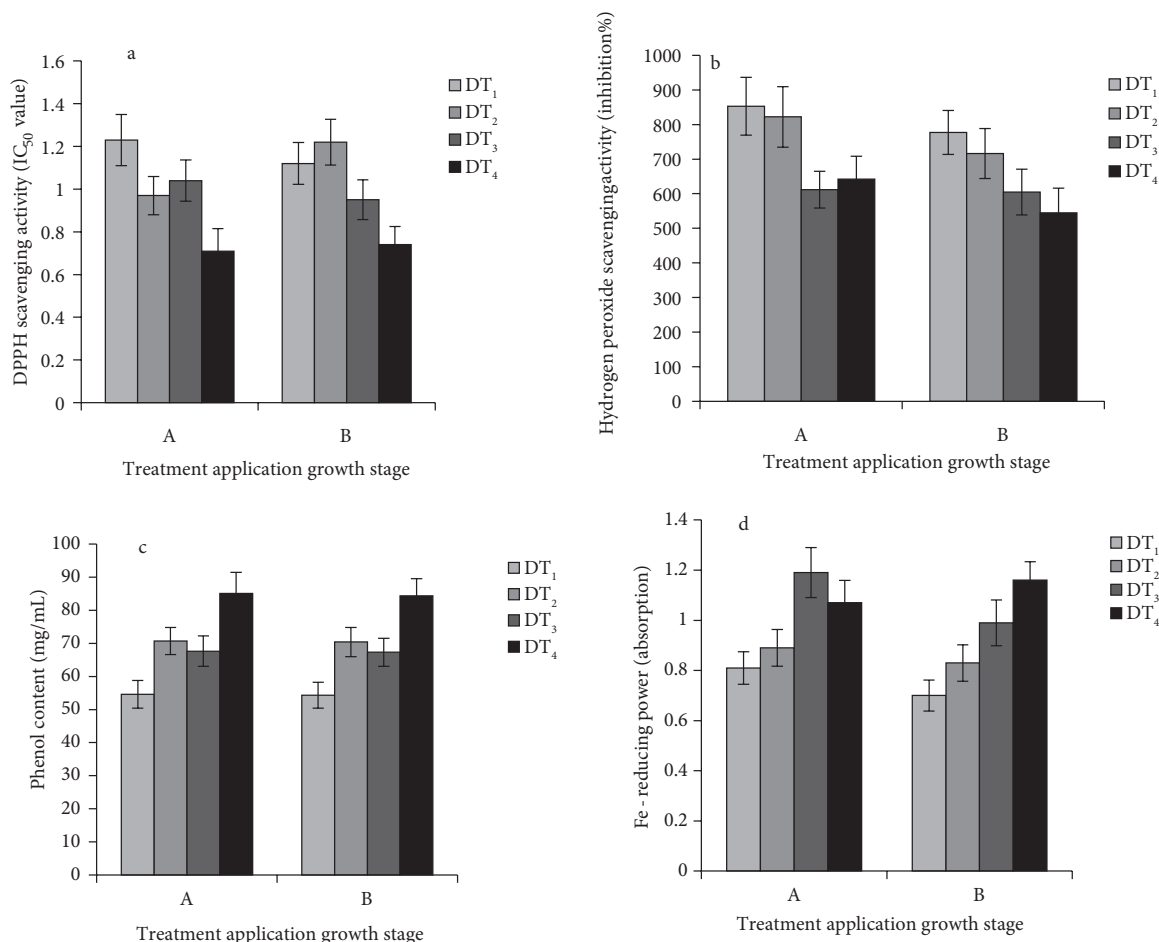


Figure 4. Antioxidative properties of *Bunium persicum* plants including DPPH scavenging activity (IC₅₀ value) (a), hydrogen peroxide scavenging activity (% inhibition) (b), phenol content (mg/mL) (c), and Fe-reducing power (absorption) (d) under different drought levels (DT₁ = 60, DT₂ = 90, DT₃ = 120, and DT₄ = 150 mm evaporation from evaporation basin) and growth stages (A = stem elongation stage and B = reproductive stage). Data are the mean values of 3 replications ± SE, represented by the vertical bar in each graph.

compounds were recognised in all samples. γ -Terpinene had the highest percentage (40.3%–42.5%) and γ -terpinen-7-al (13.9%–16%) and p-cuminaldehyde (14.9%–14.9%) had higher percentages than other compounds. P-cymene (4.5%–5.5%), 1,8-cineole (3.4%–4.2%), and limonene (3%–3.3%) were the other chemical compounds with high relative percentages.

Compounds detected in plant essential oil grown under drought conditions after the flowering stage were similar to a great extent. In general, 26 constituents were recognised, and 22 constituents were detected in all samples. Similar to previous samples, γ -terpinene had the highest percentage (39.2%–43.1%). γ -Terpinen-7-al (13.2%–16.1%) and p-cuminaldehyde (14%–15.1%) also had higher percentages among the constituents.

Chemical composition of essential oil of *Bunium persicum* was studied by some researchers. Azizi et al. (2009) analysed essential oil constituents of *Bunium*

persicum under wild and agronomical conditions, and no significant difference was observed. In general, essential oil constituents are not significantly affected by environmental stresses.

4. Conclusions

Applied treatments affected *Bunium persicum* growth, productivity, and physiological performance. In addition to the acceptable tolerance of *Bunium persicum* plants under medium and severe drought conditions in regards to yield and yield components, antioxidant activity assessment of seeds showed that this plant is a highly valuable source of natural antioxidants. With respect to the achieved results, *Bunium persicum* is tolerant to drought conditions. As a result, it is an appropriate plant for cultivation in arid and semiarid regions. Further investigations to evaluate other physiological and agronomical aspects of this plant in order to facilitate the domestication process are recommended.

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