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PHYSIOLOGICAL REACTION AND CHEMICAL COMPOSITION OF *Stachys schtschegleevii* Sosn. ESSENTIAL OIL UNDER WATER DEFICIT

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ABSTRACT

Stachys schtschegleevii Sosn. is an endemic medicinal plant belonging to the Lamiaceae family and mainly grown in North-western Iran. Drought stress is an important factor in reducing the yield of medicinal herbs. Water-stress tolerance involves subtle changes in cellular biochemistry. It appears to be the result of the accumulation of compatible solutes and of chemical compositions that can be rapidly induced by osmotic stress. For this purpose, the effect of different irrigation regimes (well-watered and irrigation after depletion of 40% and 70% of field capacity (FC)) were studied in *S. schtschegleevii*. The experiment was conducted in a randomized complete block design in three replications. The results showed that water-deficit had negative effects on shoot dry matter, relative water content, and photosynthetic pigments of the exposed plants. The essential oil (EO) content under water-deficit had an increasing trend. Water-deficit significantly increased total phenol content, proline, H_2O_2, and malondialdehyde contents. Linalool, β-pinene oxide, α-campholenal and germacrene-D were the major compounds of essential oils (EOs) affected by water-deficit stress. Finally, although water deficiency reduces the shoot dry matter yield of the *S. schtschegleevii,* the accumulation of EO increased as a plant response to water-deficit stress.

Key words: α-campholenal, dry matter, growth, drought stress, medicinal plants

INTRODUCTION

Stachys schtschegleevii Sosn. is one of the important medicinal plant of the Lamiaceae family and native to Iran, with abundant distribution in the Northwest of the country where it is known as "Arasbarani Sonbol" or "Poulak" [Mozaffarian 1966], with various medicinal properties such as antibacterial, antimicrobial, anti-asthma, anti-sinusitis, anti-cold, anti-rheumatism, anti-inflammatory, urinary tract disinfection, and treatment of respiratory tract infections. This plant is known as natural penicillin. Its most important active ingredients are flavonoids and EOs, while α-pinene, β-pinene, ocimene, limonene, and β-myrcene are the most commonly identified compounds in its EO, found in fresh leaves as well as herbaceous and flowering branches of the plant [Sonboli et al. 2005, Hazrati et al. 2020].

The amount of water available to the plant is an key climatic factor that affects the distribution of plants worldwide and can cause morphological, physiological, and biochemical alterations in the plant. Drought and salinity are two important problems for agricultural development in Iran; therefore, it is crucial to select suitable species for arid and semi-arid areas. In this regard, many indigenous farmers in these areas have chosen to grow adapted plants. Different plant species show a wide range of drought resistance due to

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their physiological, morphological, and biochemical adaptations. Morphological factors such as biomass weight, number of branches, and root dry weight can affect drought resistance [Thomas and Gausling 2000, Kapoor et al. 2020]. Some physiological changes of drought-resistant plants, including hairs on the leaf surface, osmotic regulation, tolerance to dehydration etc., are also very effective in this regard [Yang et al. 2021].

Cultivation of medicinal plants such as *S. schtschegleevii* is essential for medicinal usage and also conserving genetic resources in natural habitats. Water shortage is a serious issue affecting many parts of the world, including Iran. Studies show that photosynthesis and transpiration decrease due to water deficiency [Kapoor et al. 2020, Yang et al. 2021]. In contrast, increasing the content of EOs and phenolic compounds in *S. schtschegleevii* leads to increased pharmaceutical effects. Plant secondary metabolites are derived from primary metabolites produced through various physiological alterations. Secondary metabolites cause a significant improvement in plant growth and survival under different environmental stresses [Kliebenstein 2013]. Many studies show that plants exhibit changes in biosynthesis and accumulation of secondary metabolites against abiotic stresses [e.g. Isah 2019].

Abiotic stresses, including drought, lead to extensive metabolic changes in plant cells, ranging from synthesizing of small amounts of specific metabolites to significant changes in the composition of primary metabolites, physiological responses, and amounts of secondary metabolites. Sugars, amino acids, and amines accumulate in different plant species during drought stress conditions [Taji et al. 2002]. These metabolites, such as osmolytes or antioxidants, contribute to an increased tolerance to drought [Bartels and Sunkar 2005]. Drought stress causes different changes in the physiological traits of the medecinal plants [Mohammadi et al. 2018]. EOs are volatile secondary metabolites of plants that are applied in food, cosmetics, and pharmaceuticals. In recent years, using natural substances has increased because of concerns regarding the use of certain chemicals; however, it requires a high degree of precision [Baczek et al. 2016].

To date, no research has investigated the influence of water-deficit stress on morphophysiological and phytochemical parameters of *S. schtschegleevii*. The present study assumes that: 1) the growth and yield of *S. schtschegleevii* decreases under water-deficit; 2) the content and constituents of the EO of the *S. schtschegleevii* change under water-deficit. Given the importance of medicinal plants in ensuring community health and considering the drought stress impacts on growth and yield and changes in the active ingredients of medicinal plants, the present study concentrated on the following objectives: 1) evaluation of water-deficit tolerance of *S. schtschegleevii*; 2) investigation of the impact of water-deficit on growth and physiological characteristics of *S. schtschegleevii*; 3) evaluation of phytochemical reactions of *S. schtschegleevii* to water-deficit.

MATERIALS AND METHODS

Plant materials, experimental setup, and treatments. This experiment was performed in a commercial greenhouse located in Kalaybar in 2019, in a randomized complete block design with three replications. Experimental treatments included water-deficit stress at three levels (well-watered, irrigation after depletion 40% and 70% of field capacity (FC)) on *Stachys schtschegleevii* (Fig. 1). Seeds of plants were collected from Kaleybar (Herbarium code ASMUH0030 is stored in the herbarium of Azarbaijan Shahid Madani University, identified by Dr. Mostafa Ebadi, stored) and then the seeds were first transplanted in a bed of peat moss and cocopeat (1 : 2) in a 72-cell (30 cc) seedling tray. After reaching the desired growth of plants up to 4 and 5 leaf stages, each of the seedlings were transferred into plastic pots with an opening diameter of 20 cm and a height of 30 cm and a capacity of 10 kg. The soil analysis showed that the soil texture was sandy loam (EC = 1.165 dS m^{-1}; pH 7.9; organic matter 1.38%).

The pots were kept in the greenhouse for 16 h of light and 23°C and 8 h of darkness at 17°C. After weighing the pots they were kept in well-watered condition until the elongation of stem phase. Before flowering, water-deficit stress was imposed with watering shortage until the soil moisture content was about 70% and 40% of field capacity (assessed by Time-domain reflectometry soil moisture sensors (PMS-714, Lutron, Taipei, Taiwan) of a 15-cm probe). However, non-stressed pots were maintained at soil water content to approximately 90% and continually irrigated normal-

Fig. 1. *S. schtschegleevii* plants subjected to different water regimes

ly through the entire experimental period. The pots weighed every 1–2 days for monitoring water use and adjusting watering as plant demand enhanced. Also, fourpots withoutplant were used for determining the evaporation.

At the flowering stage of the plantsrelated to each treatment were harvested and then the aerial part was removed, packed and transferred to laboratory in a cool, ice-filled container. In the laboratory, the collected samples were divided into two fresh and dry weight samples in order to study the physiological traits in equal numbers. Fresh samples were packed into a zipper and then frozen at –20°C. The dried samples were transferred to thelaboratory for drying in an environment free of pollution and direct sunlight (in shady conditions). In this experiment, shoot dry matter, chlorophyll content, relative leaf water content, proline content, malondialdehyde content, hydrogen peroxide content, total phenol content, EO content and EO compounds were measured.

Essential oil extraction. EO was isolated using the hydro-distillation method. Then, the samples were crushedto reduce the surface area for easy volatilization of the EO. Forty grams of dried plants were added to distilled water in a flask by the Clevenger apparatus extraction method in three replications based on an approach reported by British Pharmacopoeia (1993) [British Pharmacopoeia Commission 1993]. After being boiled, samples were maintained at the minimum required temperature for boiling. After distillation, the EO was recovered directly, using a micro-pipette from above the distillate to calculate the EO content. Samples were finally stored in vials at 4°C for future analysis.

Gas chromatography and gas chromatography–mass spectrometry analysis. The composition of the EO of *S. schtschegleevii* was analyzed by gas chromatography (GC) and GC methods coupled with mass spectrometry (GC/MS). GC/MS assessment was performed using an Agilent Technologies-5975 C-MS, 7890A-GC system fitted with HP-5 MS-fused silica capillary column (30 m × 0.25 mm (ID) × 0.25 μm (FT)). The following program was used to schedule the oven temperature: from 60 (held isothermally for 2 min) to 210°C with a ramp of 3°C/min, followed by an increase to 240°C with a ramp of 20°C/min, and the final temperature maintained for 8.5 min with a run time of 60 min. The electron ionization energy of 70eV was used in the electronic ionization (EI) method, considering ion-source of 230°C, the detector temperature of 290°C MS, interface line temperature of 280°C, injector temperature of 280°C, and a split ratio of 1 : 50. EO was diluted with hexane (1 : 100 ratio), and helium was applied as carrier gas (flow rate, 1 mL/min) while consideringa mass range of 50–480 m/z and the sample injection volume of1.0 μL.

The gas chromatograph analysis was performed using an Agilent Technologies-7890A selective detector and an HP-5 fused silica capillary column (30 m × 0.32 mm (ID), 0.25 μm (FT), which included a flame ionization detector (FID). GC condition: following program used to schedule oven temperature T from 60 to 210°C with a ramp of 3°C/min followed by an increase to 240°C with a ramp of 20°C/min, and the final

temperature maintained for 8.5 min. Finally, The GC operating parameters were set at an ionization voltage of 70 eV and an ion source temperature of 200°C.

Identification of EO compositions. Individual compounds were detected and identified via comparison of their spectra andretention indices (RI) than C5-C24 n-alkanes, with mass spectra with the NIST (11.0) mass-spectral library, Wiley MS data system library (Wiley, Chichester, UK), and other studies. Normal hydrocarbons were applied at the same temperature to calculate the inhibition index. Additional identification was obtained through matching the mass spectral fragmentation patterns of various compounds with corresponding data (Adams & Wiley 7.0 library) and other published mass spectra data [Adams and Sparkman 2007]. Finally, the percentage of the constituents was assessed according to the peak areas obtained by the response factor of the detector.

Total phenolic. Total phenol content was assessed from methanolic extracts using folin by spectrophotometer at 765 nm. A standard curve prepared by gallic acid in different concentrations was used to determine the concentration of phenolic compounds, and the absorbance values were replaced in the obtained line equation. The concentration of phenolic compounds was expressed in mg GAE/g of dry weight [McDonald et al. 2001].

Relative water content. The leaf relative water content (RWC) was determined using fully developed young leaves. They were rapidly sealed into clear plastic bags, and the fresh weights (FW) were recorded immediately after transferring the samples to the laboratory. Then, they were submerged in double distilled water within the covered Petri dish for six hours at room temperature, and the turgid weight (TW) was recorded. The leaf samples were oven-dried at 70°C for 48 hours, and then the dry weight (DW) was determined. Ultimately, the following formula was considered:

$$RWC\ (\%) = (FW - DW)/(TW - DW) \cdot 100$$

Plastid pigment measurements. Shoot fresh tissues (0.1 g each sample) were grounded in 5 mL of acetone (80% v/v) to extract photosynthetic pigments (i.e. chlorophyll (Chl) *a*, Chl *b*, and carotenoids). The sample absorbance was measured at 645, 663, and 470 nm in a T80+ UV–Vis spectrophotometer (PG Instrument Ltd., UK) [Lichtenthaler and Wellburn 1983].

H_2O_2 concentration determination. In order to determine H_2O_2 content in the shoot of *S. schtschegleevii* plants, 0.1 g fresh tissues were homogenized using 5 ml of 0.1 % w/v trichloroacetic acid (TCA) and centrifuged (12,000 × *g* for 15 min). Then supernatant (0.5 ml) was supplemented to 0.5 ml of potassium phosphate buffer (10 mM, pH 7.0) and 1 ml of potassium iodide (1 M). The upper phase was aliquoted to read its absorbance at 390 nm. H_2O_2 was used for graphing calibration curve in order to calculate H_2O_2 concentration [Velikova et al. 2000]. Based on the standard curve, the H_2O_2 content was expressed as $\mu molg^{-1}$ FW.

Assessment of MDA content. Shoot fresh tissues (0.1 g each) were crushed and blended in 5 mL of TCA solution (0.1 % w/v) and centrifuged (12,000 × *g* for 15 min). Two millilitres of the supernatant was added to 2 mL of TBA (0.6 % w/v). The mixture incubated for 30 min, at 95°C; the samples were cooled down on the ice and were centrifuged (4,000 × *g* for 20 min). The absorbance of the supernatant was measured at 532 nm. The amount of MDA calculated based on Heath and Packer 1968]. The MDA content was calculated using a correction factor of 155 mM^{-1} cm^{-1} and expressed in terms of $nmoleg^{-1}$ FW.

Data analysis. The experimental procedures were done in triplicate. Analysis of variance (one-way ANOVA) using Statistical Analysis System (SAS) software 9.2 (SAS Institute, Cary, NC, United States) was applied for evaluation of the differences between the treatments, and the graphs were drawn by Excel 2013 software. For mean comparison, Duncan's multiple range test (DMRT) was regarded as significant at $P \leq 0.05$.

RESULTS

Shoot dry weight. The results showed that water-deficit stress had a significant effect on the shoot dry weight of *S. schtschegleevii* plants (Tab. 1). Water stress significantly decreased the shoot dry weight with the increase in water stress intensity while; there was no significant difference between the mean shoot dry weight of well-watered and after depleting 40%

Table 1. Analysis of variance (ANOVA) for studied traits in *S. schtschegleevii* plants under water-deficit stress

Traits	Mean square		
	block	water regimes	error
Shoot dry weight	0.007^{ns}	0.208^{*}	0.025
RWC	1.358^{*}	7.684^{**}	0.089
Chlorophyll *a*	0.00001^{ns}	0.00030^{*}	0.00004
Chlorophyll *b*	0.000002^{ns}	0.000033^{*}	0.000004
Total chlorophyll	0.00002^{ns}	0.00055^{*}	0.00007
Carotenoids	0.00006^{ns}	0.00057^{**}	0.00003
Malondialdehyde	0.0009^{ns}	0.0125^{*}	0.0010
Hydrogen peroxide	0.00024^{ns}	0.00060^{*}	0.00004
Proline	7.611^{*}	51.719^{**}	0.821
Total phenol	805.94^{**}	6340.01^{**}	19.97
EO content	0.0003^{ns}	0.0116^{**}	0.0001

*,** Significantly different at the 1 and 5% probability level, respectively, ns not significant

treatment. Irrigation after 70% depletion of FC significantly reduced the shoot dry weight than well-watered and after depleting 40% treatment (Fig. 2).

Relative water content. The results showed that plant RWC of *S. schtschegleevii* affected by different water treatment levels (Tab. 1). Water-deficit stress significantly decreased RWC content in *S. schtschegleevii*. The mean comparison of data showed a decrease of 1.41 and 2.66 % in plant leaf after depleting 40 and 70% of FC, respectively, than the control treatment (Fig. 2).

Photosynthetic pigments concentaration. Water treatment levels significantly affected the content of chlorophyll *a*, *b*, total chlorophyll, and carotenoids in *S. schtschegleevii* (Tab. 1). Comparison of mean data showed a decrease of 7.7% and 11.0% in chlorophyll *a*, 10.8% and 14.9% in chlorophyll *b*, and 8.3% and 11.9% in total chlorophyll content in plant leaf after depleting 40 and 70% of FC, respectively. There was no significant difference ($P \leq 0.05$) between the mean chlorophyll *a*, *b*, total chlorophyll contents of well-watered and after depleting 40% treatment (Fig. 3). Compared to the well-watered plants, an increase of 9.9%, and 9.3% in the content of carotenoids was observed in conditions of water-deficit stress at 40 and 70% depletion of FC, respectively (Fig. 3).

H_2O_2 and MDA content. In this experiment, water availability had the greatest influence on the H_2O_2 and MDA content in *S. schtschegleevii* (Tab. 1). H_2O_2 content increased by 7% and 23%, and MDA level increased by 50% and 33% in water-deficit conditions with 40 and 70% depletion of FC, respectively than the control treatment (Fig. 4).

Proline content. Resaults showed that water stress significantly affects proline accumulation in *S. schtschegleevii* ($P \leq 0.05$), with the proline content of plant gradually increasing as the water levels decreased (Tab. 1). There was an increase of 9% and 18% in proline content during water-deficit stress conditions with 40% and 70% depletion of FC, respectively compared to well-watered plants (Fig. 5).

Total phenolic content. Resault showed that water stress significantly affects on the total phenol content in *S. schtschegleevii* ($P \leq 0.05$) (Tab. 1), water-deficit stress conditions led to a significant increase in the total phenol content in *S. schtschegleevii* (Fig. 6). Water stress had different effects on the total phenol content. The contentration of total phenols under mod-

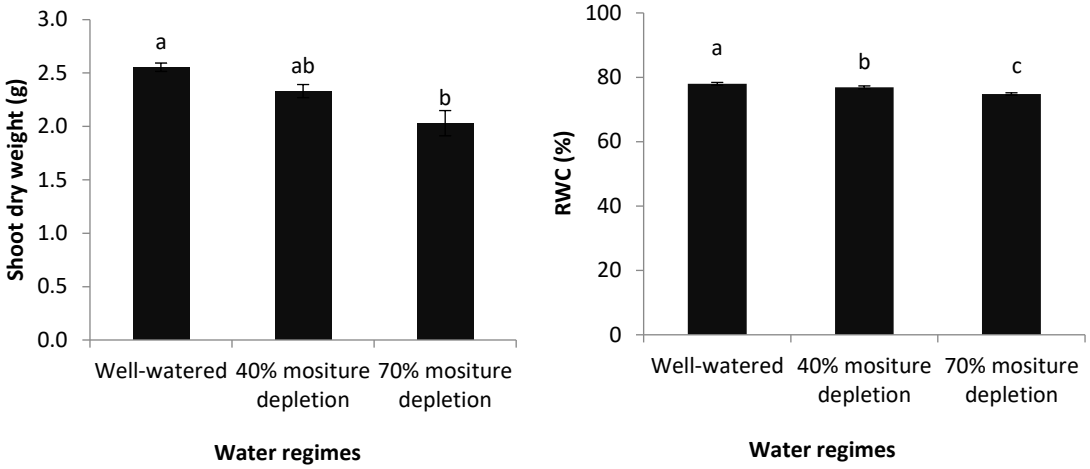

Fig. 2. Variation of shoot dry weight and leaf relative water content (RWC) in *S. schtschegleevii* plants subjected to water-deficit stress. Different letters indicate significant differences between treatments at $P \leq 0.01$ based on Duncan's Multiple Range Test (DMRT); vertical bars indicate standard deviation (n = 3)

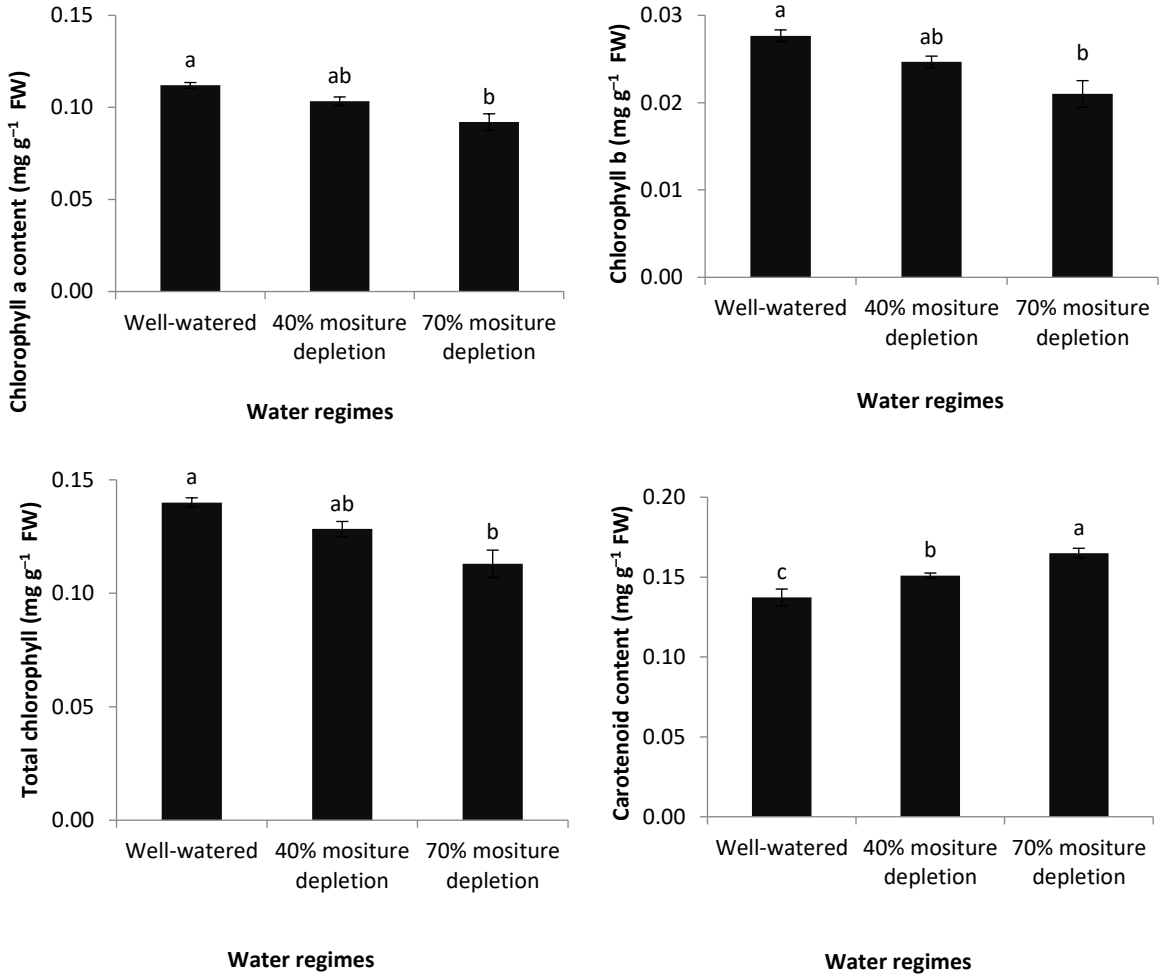

Fig. 3. Variation of photosynthetic pigments, chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content in *S. schtschegleevii* plants subjected to water-deficit stress. Different letters indicate significant differences between treatments at $P \leq 0.01$ based on Duncan's multiple range test (DMRT); vertical bars indicate standard deviation (n = 3)

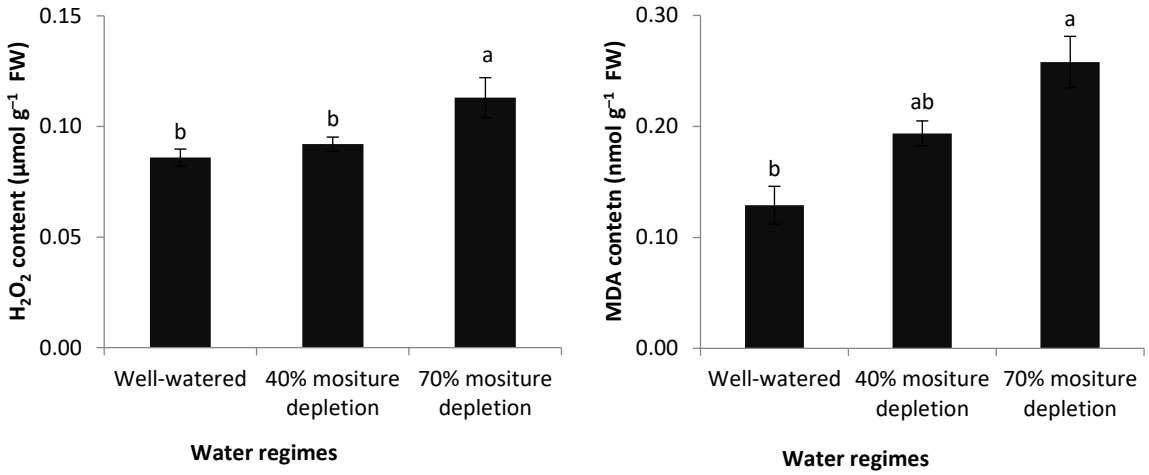

Fig. 4. Changes in peroxide hydrogen (H_2O_2) and malondialdehyde (MDA) contents in *S. schtschegleevii* plants subjected to water-deficit stress. Different letters indicate significant differences between treatments at $P \leq 0.01$ based on Duncan's multiple range test (DMRT); vertical bars indicate standard deviation (n = 3)

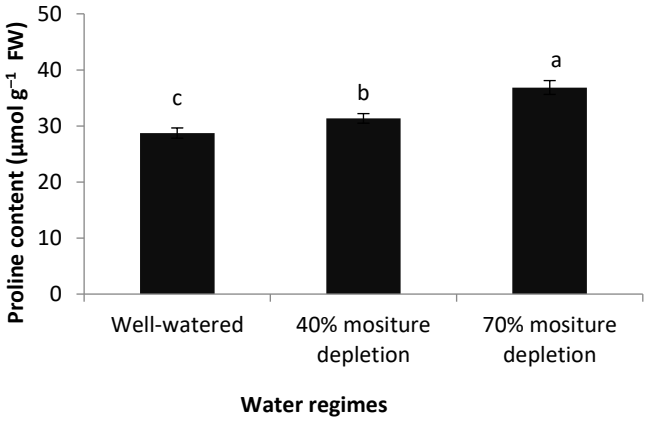

Fig. 5. Variation of proline content in *S. schtschegleevii* plants subjected to water-deficit stress. Different letters indicate significant differences between treatments at $P \leq 0.01$ based on Duncan's multiple range test (DMRT); vertical bars indicate standard deviation (n = 3)

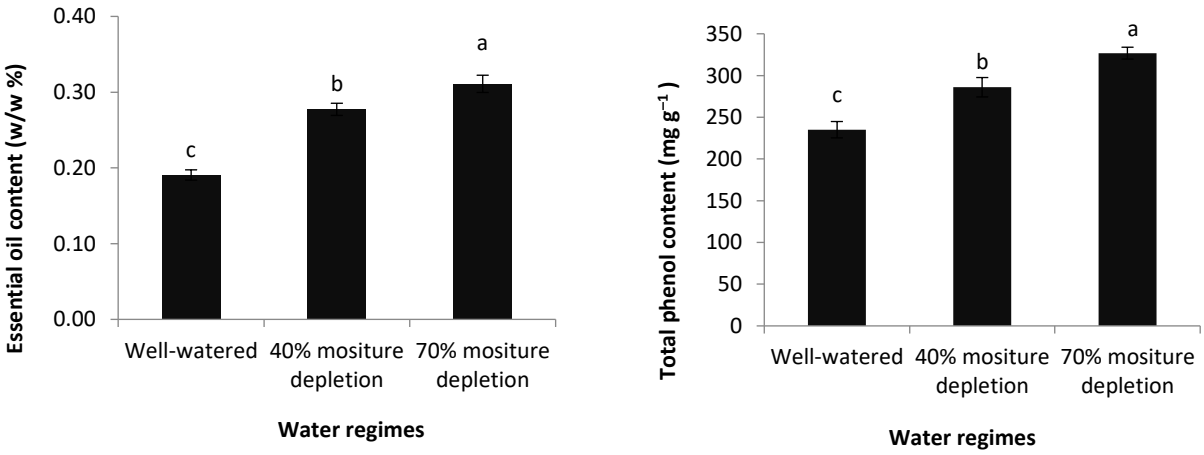

Fig. 6. Changes EO content and total phenolics in *S. schtschegleevii* plants subjected to water-deficit stress. Different letters indicate significant differences between treatments at $P \leq 0.01$ based on Duncan's multiple range test (DMRT); vertical bars indicate standard deviation (n = 3)

Table 2. Composition of essential oils (%) in *S. Schtschegleevii* plants under different water-deficit stress conditions

Compounds	RI	LRI	Water regimes		
			well-watered	40% moisture depletion	70% moisture depletion
α-Thujene	928	924	1.99 ±0.11	1.87 ±0.13	2.64 ±0.56
α-Pinene	**953**	**932**	**5.31 ±0.62**	**2.13 ±0.19**	**1.34 ±0.14**
β-Pinene	978	974	0.75 ±0.02	1.67 ±0.15	1.15 ±0.14
cis-Pinane	982	982	0.37 ±0.03	0.41 ±0.03	0.56 ±0.05
p-Mentha-1(7),8-diene	1003	1003	0.81 ±0.04	1.22 ±0.04	1.05 ±0.10
1,4-Cineole	1011	1012	0.19 ±0.01	0.21 ±0.02	1.74 ±0.10
α-Terpinene	1016	1014	1.48 ±0.12	2.26 ±0.21	0.25 ±0.02
(Z)-β-ocimene	1035	1032	0.19 ±0.02	0.28 ±0.03	2.54 ±0.21
(E)-β-ocimene	1039	1044	0.11 ±0.01	0.11 ±0.02	2.80 ±0.18
cis-Linalool oxide	1072	1067	0.68 ±0.03	0.33 ±0.03	1.48 ±0.16
Terpinolene	1080	1086	2.49 ±0.17	0.27 ±0.02	0.31 ±0.04
Linalool	1105	1095	2.00 ±0.19	2.88 ±0.12	4.16 ±0.89
trans-thujone	1113	1112	0.93 ±0.03	2.09 ±0.10	0.35 ±0.03
α-Campholenal	**1126**	**1122**	**45.15 ±2.15**	**32.40 ±2.10**	**36.60 ±2.08**
(Z)-Epoxyocimene	1129	1129	0.41 ±0.02	0.35 ±0.02	0.36 ±0.03
cis-p-Mentha-2,8-dien-1-ol	1133	1133	0.32 ±0.03	0.28 ±0.01	0.50 ±0.02
β-Pinene oxide	**1152**	**1154**	**9.18 ±0.67**	**1.79 ±0.10**	**4.65 ±0.32**
3-Thujanol	1163	1164	0.50 ±0.03	18.51 ±0.94	0.42 ±0.02
Verbanol	1200	1197	0.38 ±0.03	0.26 ±0.02	0.57 ±0.03
p-Cymen-9-ol	1207	1204	0.27 ±0.01	0.36 ±0.03	0.87 ±0.04
trans-Pulegol	1214	1213	0.32 ±0.02	0.19 ±0.02	0.59 ±0.02
p-Mentha-1,4-dien-7-ol	1322	1325	1.34 ±0.12	0.17 ±0.02	0.23 ±0.01
Sesquithujene	1402	1405	0.24 ±0.02	0.29 ±0.02	0.29 ±0.02
Germacrene D	**1446**	**1484**	**19.47 ±1.12**	**26.40 ±1.87**	**30.01 ±1.98**
δ-Cadinene	1524	1522	1.00 ±0.09	0.81 ±0.03	0.59 ±0.04
trans-Isolongifolanone	1626	1625	0.80 ±0.04	0.56 ±0.02	1.11 ±0.11
14-Hydroxy-(Z)-caryophyllene	1664	1666	1.13 ±0.11	0.53 ±0.02	0.90 ±0.08
Khusinol	1677	1679	0.33 ±0.03	0.25 ±0.01	0.53 ±0.03
epi-Alpha-bisabolol	1680	1683	0.36 ±0.04	0.18 ±0.01	0.43 ±0.02
Iso-Longifolol	1730	1728	0.61 ±0.03	0.52 ±0.03	0.32 ±0.01
Total of compounds identified (%)			99.12	99.52	99.36
Classes of constituents					
Monoterpene hydrocarbons			13.50	10.23	12.64
Oxygenated monoterpenes			61.68	59.78	52.54
Sesquiterpene hydrocarbons			20.70	27.49	30.90
Oxygenated sesquiterpene			3.23	2.02	3.29

RI – retention index, LRI – literature retention index

erate and severe stress significantly increased, while in well-watered plants decreased. The total phenol level increased by 14% and 22% in water-deficit conditions at 40 and 70% depletion of FC, respectively, compared to well-watered plants (Fig. 6).

Essential oil content. Water availability affected on the content of EO (Table 1). The result showed that water deficit increased the EO level of *S. schtschegleevii* (Fig. 6). Significant increases in EO content of *S. schtschegleevii* by 12% and 45% were observed after irrigation depleting at 40 and 70% of FC, respectively, compared to well-watered plants (Fig. 6).

Essential oil compositions. According to the findings of the assessment of *S. schtschegleevii,* EO compounds determined with GC/MASS, 30 types of compounds were identified at the flowering stage. Table 2 shows the effect of soil moisture levels on the chemical composition of EO extracted from *S. schtschegleevii*. The major compounds of EOs were linalool, -β-pinene oxide, α-campholenal and germacrene-D (Tab. 2). The results showed linalool (1.99%), pinene oxide-β (9.18%), α-campholenal (45.15%), and germacrene-D (19.46%) in well-watered conditions. Linalool (2.87%), β-pinene oxide (1.78%), α-campholenal (32.39%), and germacrene-D (26.40) contents were reported in the plant after depleting 40% of FC. Similarly, after depleting 70% of FC, the contents of linalool (4.16%), β- pinene oxide (4.65%), α-campholenal (36.60%), and germacrene-D (30.01) were reported. Significant increases of 35.61% and 54.15% in germacrene-D content were observed under water stress conditions with 40% and 70% depletion of FC in *S. schtschegleevii*, respectively (Tab. 2).

DISCUSSION

Results showed that irrigation after 70% depletion of FC reduced the shoot dry weight. The dry weight loss of the plant under water-deficit stress could be related to the reduction in cell elongation and expansion due to a reduction in turgor pressure, as plant cell growth is the most sensitive physiological process due to the water deficit, also, the reduction of photosynthesis, which is mainly due to the change in stomatal regulation [Cornic 2000, Fahad et al. 2017]. Cell shrinkage because of drought stress increases osmotic potential, which can eventually lead to aggregation, denaturation of proteins, and inhibition of the normal function of enzymes involved in photosynthesis [Hoekstra et al. 2001].

Water-deficit stress reduces the turbidity and water potential in plants, and as a result, affects different physiological processes negatively [Lisar et al. 2012]. Also, water-deficit stress causes significant changes in plant biochemical processes by inhibiting photosynthesis and reducing growth [Kapoor et al. 2020].

Photosynthetic pigments can be damaged in plants during intense stress conditions that subsequently lead to the production of reactive oxygen species (ROS) and and impairs the performance of important enzymes [Slama et al. 2007]. Chlorophyll content in plants is one of the important factors in maintaining photosynthetic capacity. In the present experiment, under water-deficit stress, it led to a significant reduction in chlorophyll content of *S. schtschegleevii*. It seems that the decrease in chlorophyll under water stress is due to the increase in the production of oxygen free radicals, which cause peroxidation and thus increase the activity of the chlorophyll enzyme under stress, which leads to the decomposition of chlorophyll [Hazrati et al. 2016]. Plants under severe water stress may also reduce chlorophyll to affect the oxidative stress of the photosynthesis at which time the photosynthesis stops due to excessive light energy [Aranjuelo et al. 2011]. Furthermore, the amount of damage to chlorophyll *a* was higher than chlorophyll *b*. Other researchers have also reported the susceptibility of chlorophyll *a* to chlorophyll *b* under water stress [Sayyad-Amin et al. 2016]. The results of the present study are consistent with the results of research conducted by Minaei et al. 2019 on the Marjoram; which reported that under severe drought stress significantly decreased chlorophyll content. In addition to their role as adjuvant pigments, carotenoids contribute as antioxidants and free radical scavenging factors in plants under moderate stress conditions [Pérez-Gálvez et al. 2020].

Studies show that partial closure of the stomata that occurs against water scarcity, as a water conservation method, also limits the entrance of CO_2 as well as its availability for photosynthesis. In addition to decreasing the efficiency of rubisco carboxylation, oxygenation increases simultaneously, resulting in increased energy losses and ROS generation [Noctor et al. 2002]. Decreased photosynthesis or CO_2 fixation leads to a

reduction in the reproduction ofnicotinamide adenine dinucleotide phosphate(NADP+), the final electron transport chain (ETC) electron receptor in chloroplasts. Excessive reduction of ETC, therefore, results in the electron transfer to O_2 and subsequent ROS production [Smirnoff 1993]. An imbalance between light absorption and its use in photosystem II contributes to alterations in the photochemistry of chloroplasts in leaves under drought stress, leading to overproduction of highly reactive and dangerous ROS species [Foyer and Noctor 2000]. High production of ROS results in oxidative damage, such as lipid peroxidation of cell membranes or even cell death [Sachdev et al. 2021].

Among the various biochemical reactions to water stress, proline accumulation is commonly observed in plants [Hayat et al. 2012]. It is considered water stress and increases the resistance of plants to adverse environmental conditions, prevents the change like proteins and promotes the protection and stability of cell membranes [Claussen 2005]. Researchers report that proline is one of the the most important stable amino acid under non-biological stress conditions. Our experiment shows that under water stress conditions the amount of water in the tissues decreases and causes *S. schtschegleevii* to increase the resistance to stress, and increases the accumulation of proline in its tissues. High proline production helps plants to maintain low water potential and derive water from the environment under stressful conditions [Hayat et al. 2012].

Water-deficit stress had a positive effect on total phenol content. Reports have also shown an increase in the internal content of the total phenol of the plants against drought stress in different medicinal plants. Changes in the amount of phenol content in plants during abiotic stress could be linked to ROS generation due to the role of flavonols in plant defense. For example, water-deficit stress increased phenolic compounds and photosynthetic pigments while decreased the fresh and dry weight of *Trachyspermum ammi* [Azhar 2011]. The phenolics contents in *Hypericum brasiliense* and *Artemisia accumulation* increased under water-deficit stress conditions [Verma and Shukla 2015]. Concentrations of secondary metabolites such as hyperforin, hypericin, and pseudohypericin increased [Zobayed et al. 2007]. As stated by Mohammadi et al. [2021], caffeic acid and rosmarinic acid contents, in the shoot extract of *Melissa officinalis* increased under water-deficit stress. Due to the correlation between increasing the activity of phenylalanine ammonia-lyase (PAL) and increasing the concentration of phenols, it has been reported that the amount of biosynthesis of these compounds in drought stress plants increases sharply compared to normal irrigation plants. PAL enzyme activity is possible under stress conditions as the beginning of cell adaptation processes against dehydration [Jaafar et al. 2012].

Increase of EO percent under water stress may be due to its posetive effect on oil biosynthesis. Studies showed that water acts as an important agent for plants because it directly affects the transport of nutrients and metabolites in various plant components. Lack of water or high transpiration rate can induce drought stress in plants, affecting the generation of secondary metabolites. Increasing the generation of some secondary metabolites in plants will also be useful in inducing drought tolerance [Verma and Shukla 2015]. Water-deficit stress in *Ocimum americanum* and *Ocimum basilicum* caused a significant effect on different nutrient concentrations, proline, carbohydrates, and plant EO content [Khalid 2006].

The changes in EO compositions under abiotic stress conditions may be due to the changes in enzyme activity and plant metabolism [Hussain et al. 2018]. Terpene synthases are the main gatekeepers engaged in terpene biosynthesis. Water-deficit stress upregulates terpenes (e.g., monoterpene, diterpenes, and sesquiterpene) and their biosynthesis [Kumar et al. 2021]. A study also found thatwater availibility affected the quantity and quality of EO components more than other environmental factors [Szabó et al. 2020]. In the present experiment, the predominant compounds showed variability under water stress conditions, for example, the amount of α-campholenal decresed while germacrene-D significantly increased. Water stress affected bothcompounds concentration and the biosynthesis of the main constituents (germacrene-D) with pharmacological value. The findings discussed above show that changes in EO content and compositions in plants arean adaptive response to water-deficit stress tolerance.

CONCLUSIONS

There is a very limited research work available concerning the effects of water-deficit stress on

morphophysiological and phytochemical traits of *S. schtschegleevii*. Therefore, our result indicated for the first time that cultivation of *S. schtschegleevii* plants resulted in a significant reduction in the shoot dry matter, leaf relative water content and photosynthetic pigments of *S. schtschegleevii* plants due to water deficit stress. Also, water-deficit stress significantly increased H_2O_2 and MDA contents. The total phenol content enhanced during water deficit stress. Water-deficit stress significantly increased proline content in plants as well. Considering that the aim of the study on the cultivation of *S. schtschegleevii* plants is to identify the effect of water-deficit stress for improving the content and quality of EO as well. Therefore, not only water-deficit stress resulted in increase EO content but also treatment in irrigation after depleting 40 and 70% of FC resulted in improvement of EO composition in *S. schtschegleevii*. Water-deficit stress under both conditions influenced the major compounds of EOs including linalool, β-pinene oxide, α-campholenal and germacrene-D. Finally, water-deficit stress plays a special role in enhancing the EO contents and quality as well.

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