

Research paper

## Changes in growth and essential oil content of dill (*Anethum graveolens*) organs under drought stress in response to salicylic acid

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### Abstract

Some of the harmful impacts of water shortage on crop performance may be alleviated by growth regulators such as salicylic acid. So, an experiment was arranged as a split-plot design based on randomized complete blocks in three replicates to assess changes in essential oil content of dill (*Anethum graveolens* L.) organs in response to water availability (water supply after 70, 100, 130, 160 mm evaporation as normal watering and mild, moderate and severe stresses, respectively), and salicylic acid (SA) levels (water spray and 0.6, 1.2 mM SA). Irrigation levels and salicylic acid treatments were assigned to the main and sub-plots, respectively. The results showed that chlorophyll a and especially chlorophyll b decreased with increasing drought stress. The ground green cover and plant organ masses (leaves and stem, flowers, and seeds) were only decreased under severe water deficit. Essential oil percentage of dill organs increased with increasing water deficit up to moderate stress, but thereafter it was decreased as water deficit severed. The highest essential oil yield of the vegetative parts and flowers was also produced in moderately stressed plants, but the greatest essential oil yield of seeds was recorded under mild water deficit. Exogenous salicylic acid enhanced the essential oil percentage of all dill organs, especially under moderate water limitation. The essential oil yield of dill organs was also increased by the salicylic acid treatment. The application of 1.2 mM salicylic acid was more effective in improving essential oil production of dill.

**Keywords:** Chlorophyll; Dill; Essential oil; Green cover; Salicylic acid; Water stress

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### Introduction

Dill (*Anethum graveolens* L.) is an important aromatic and medicinal plant (Valdiani *et al.* 2012), characterized by high essential oil content (Dyduch 2000), particularly in flowers and seeds (Ghassemi-Golezani *et al.* 2008; Ghassemi-Golezani and Nikpour-Rashidabad 2017). The major essential oil constituents of dill are carvone, limonene, and phellandrene. The other important constituents are pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin, myristicin, myristin, apiol, and dillapiole (Kaur and Arora 2010). The growth and productivity of this plant may be

limited by adverse environmental conditions such as water stress.

Drought stress is the most limiting factor for crop growth and production (Abobatta 2019). This stress causes many changes in plants that reduce yield and quality (Ghassemi-Golezani *et al.* 2015; Fathi and Barari Tari 2016; Ghassemi-Golezani *et al.* 2018). Drought stress occurs when water availability drops to sub-optimal levels for the growth and development of plants (XU *et al.* 2010). The ground green cover and chlorophyll content of milk thistle (*Silybum marianum*) decreased with increasing drought stress (El-Sayed *et al.* 2019). Decreasing water supply was

also led to a significant reduction of plant biomass in coriander (*Coriandrum sativum*). Moreover, the biomass of individual organs of ajowan (*Trachyspermum ammi* L.) was diminished with declining water availability (Ghassemi *et al.* 2017). Drought stress also decreased shoot and total dry weight in the chickpea (*Cicer arietinum*) plants (Sohrabi *et al.* 2012). Water limitation during reproductive stages caused 50-80% reductions in grain yield of chickpea due to disruptions in photosynthesis (Ghassemi-Golezani *et al.* 2013). Some reports suggest that water shortage enhances the synthesis of secondary metabolites, antioxidant enzymes activities, and accumulation of soluble sugars (Ghassemi *et al.* 2017). The essential oil content of dill organs declined with diminishing water supply, but essential oil yield under severe stress decreased due to a decrement in the yield of different organs (Petropoulos *et al.* 2008). In comparison, when drought stress occurred during reproductive stages, both essential oil percentage and yield enhanced, particularly under mild stress (Saeedfar *et al.* 2015). According to Amiri *et al.* (2018), although seed essential oil content of dill increased with decreasing water supply, the highest essential oil yield per unit area was recorded under mild and moderate water limitations. However, severe water deficiency significantly reduced the essential oil yield due to a large decrement in seed yield. Tsamaidi *et al.* (2017) reported that the percentage of essential oil of flowers and seeds of dill increased with increasing water stress, and the highest essential

oil yield per unit area was produced under moderate water deficit.

Plant growth regulators such as salicylic acid (SA) have a key role in the systemic plant defense responses against environmental stresses. SA also regulates the biochemical and physiological processes of plants in response to stressful conditions (Rivas-San Vicente and Plasencia 2011; Farhangi-Abriz and Ghassemi-Golezani 2016). The SA treatment enhances antioxidative capacity, osmolytes, water content, membrane integrity, photosynthetic pigments, and green cover of drought-subjected rapeseed plants (Ghassemi *et al.* 2019). In this way, salicylic acid can alleviate some of the harmful effects of drought stress on the field performance of various crops (Yazdanpanah *et al.* 2011). The SA-treated plants have a higher shoot dry weight compared with untreated plants. According to Yeganehpour *et al.* (2017), the SA treatment had a minimal effect on essential oil components of coriander leaves but significantly improved the essential oil content of the seeds. In a study, the percentage of essential oil content of ajowan increased but seed and essential oil yields decreased due to drought stress. Application of SA increased seed yield and essential oil production of this medicinal plant (Ghassemi-Golezani *et al.* 2018). Therefore, this research was aimed to find out an appropriate concentration of salicylic acid to improve drought tolerance and essential oil yield of dill.

## Materials and Methods

### Experimental arrangement

This research work was carried out in 2019 at the Research Farm of the University of Tabriz, Iran (Latitude 38° 05tN, Longitude 46°17tE, Altitude 1360 m above sea level) to examine the changes in essential oil production of dill organs in response to different levels of irrigation (I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>: Irrigation after 70, 100,130, and 160 mm evaporation from the class A pan, as normal irrigation and mild, moderate, and severe stresses, respectively) and foliar spray of water (SA<sub>0</sub>) (1000 L ha<sup>-1</sup>) and salicylic acid (SA<sub>1</sub>, SA<sub>2</sub>: 0.6 and 1.2 mM, respectively). The mean annual precipitation and temperature of this region in 2018 were reported as 247.61 mm and 10.94°C, respectively. The experiment was laid out as split plots using the randomized complete block design in three replications. The irrigation and salicylic acid levels were allocated to the main plots and sub-plots, respectively. Soil samples from 0–30 cm depth of experimental area were tested for physical and chemical properties, which are presented in Table 1.

Each plot had six rows with 5 m length and 25 cm distance from each other. Seeds of dill (Tabriz ecotype) were first treated with 2 g kg<sup>-1</sup> Benomyl and then were sown in 1.5 cm depth of sandy loam soil on 12 May, 2019. Regular irrigations were carried out after sowing until the seedling establishment (BBCH-scale stage 13), and thereafter irrigation levels were regulated according to the treatments. The emerged seedlings were thinned to achieve 80 plants m<sup>-2</sup>. Weeds were removed from the plots as required. Water (control) and SA were sprayed on plants at

the vegetative (BBCH-scale stage 43) and flowering stages (BBCH-scale stage 64).

### Chlorophylls a and b

Chlorophylls a and b were estimated according to Sukran *et al.* (1998). At the full flowering stage (BBCH-scale stage 65), a cut fresh leaf sample (0.5 g) from each plot was ground and solved in 5 mL of 100% acetone. This sample was incubated in darkness at 3-5 °C for 24 h and then the absorbance of the solution was recorded at 662 nm and 645 nm, using a spectrophotometer (Model Analytik Jena Spekol 1500 Germany). The chlorophylls a and b were estimated as:

$$\text{Chlorophyll a (mg g)} = (11.75 A_{662} - 2.350 A_{645}) \times 1000$$

$$\text{Chlorophyll b (mg g)} = (18.61 A_{645} - 3.960 A_{662}) \times 1000$$

### Ground green cover

The green cover of plants in each plot was also measured at the flowering stage, using a wooden frame (50 cm × 50 cm), with 100 equal sections. The frame was placed in each plot and the sections with at least 50% green area of plants were counted. Matured plants in 1 m<sup>2</sup> of the middle part of each plot were harvested (80 plants) and the above ground biomass was oven-dried at 75 °C for 48 hours and then plant biomass per unit area was determined.

### Organs masses and Essential oil content

Vegetative organs (leaves and stems) and flowers of 30 plants at flowering, and seeds of 80 plants

Table 1. Physical and chemical properties of the experimental field soil

Depth	Soil type	Sand (%)	Clay (%)	Silt (%)	EC (dS m <sup>-1</sup> )	pH	OC (%)	Fe (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	N (%)
0-30	Sandy-Loam	74	12	14	2.92	8	0.37	2.6	255	4.9	0.04

from each plot at maturity (BBCH-scale stage 95) were harvested for the measurement of organs biomasses (leaves, stems, flowers, and seeds) and essential oil extraction. The samples were dried at room temperature of 20-25 °C for 14 days and weighed. A sub-sample of 30 g from each dill organ of each plot was mixed with 500 ml distilled water and then the essential oil content was extracted through hydro distillation at 250 °C for 3 hours, using a Clevenger glass (Shu *et al.* 2003). The essential oil content was determined as the percentage of each organ and then the essential oil yield per unit area was calculated as:

$$\text{Essential oil yield (g m}^2\text{)} = \% \text{ essential oil content} \times \text{organ yield (g m}^2\text{)}$$

The data analysis was performed by MSTAT-C and the mean values were compared by Duncan multiple range test at  $p \leq 0.05$ . The figures were drawn by Excel software.

## Results

The irrigation  $\times$  SA interaction was significant for the chlorophyll a, chlorophyll b, green cover, and vegetative and seeds masses. Irrigation and SA also had significant effects on the dry weight of flowers, with no significant interaction (Table 2).

### Chlorophylls a and b

The chlorophylls a and b of dill leaves decreased

with increasing the water shortage but the SA treatment enhance the chlorophyll content of leaves. Plants treated with SA<sub>2</sub> had the highest chlorophyll a content under all irrigation levels (Table 3). SA<sub>2</sub> also improved the chlorophyll b content under normal irrigation and severe stress (Table 3).

### Ground green cover

Percentage ground green cover of dill was significantly decreased by increasing water stress. Exogenous application of salicylic acid significantly improved green cover under all irrigation levels, especially in moderate and severely stressed plants. Foliar spray of 1.2 mM SA was the superior treatment for enhancing the green cover of dill in all stressed and unstressed plants (Table 3).

### Vegetative mass

The irrigation  $\times$  SA interaction was significant for the vegetative mass of dill plants. The mean vegetative mass of plants treated with 1.2 mM SA was significantly higher than untreated plants under stressful and non-stressful conditions. The SA advantage on vegetative mass was more pronounced under mild stress, followed by moderate stress (Table 3).

Table 2. Analysis of variance of the data for chlorophylls a, b, green cover, and organs masses of dill affected by water supply and salicylic acid

Treatments	df	Mean squares					
		Chlorophyll a	Chlorophyll b	Green cover	Vegetative mass	Flower mass	Seed mass
Replication	2	28.50	28.37	17.86	3531.12	394.10	283.94
Irrigation (I)	3	1134.11**	341.00 *	459.29*	102.73 <sup>ns</sup>	2624.33**	15843.8**
Ea	6	10.70	38.07	53.26	28.27	13.20	15.13
Salicylic acid (SA)	2	176.87 **	58.32 *	396.86**	3388.30**	49.98**	861.24**
I × SA	6	24.84 **	36.93 *	27.93*	456.68**	0.52 <sup>ns</sup>	2.54**
Eb	16	5.10	12.70	9.41	2.63	0.49	0.01
CV (%)	-	10.63	27.55	4.47	2.19	0.73	0.04

ns, \*, \*\*: not significant and significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

Table 3. Means of different traits for the interaction of water supply × salicylic acid (SA) in dill plants

Irrigation levels	SA levels	Chlorophyll a (mg g <sup>-1</sup> )	Chlorophyll b (mg g <sup>-1</sup> )	Green cover (%)	Vegetative mass (g m <sup>-2</sup> )	Seed mass (g m <sup>-2</sup> )
I <sub>1</sub>	SA <sub>0</sub>	29.90± 2.27c	20.33± 4.33b	77.00± 0.67ab	58.02± 5.45g	239.46± 4.04d
	SA <sub>1</sub>	37.29± 3.49b	15.34± 0.18bc	79.00± 1.46ab	56.02± 5.85c	241.26± 4.04c
	SA <sub>2</sub>	43.01± 0.94a	28.31± 0.27a	89.00± 2.66a	88.06± 7.33c	255.74± 4.04a
I <sub>2</sub>	SA <sub>0</sub>	18.07± 0.30de	11.58± 6.04cd	58.33± 2.56de	49.15± 11.54h	229.46± 3.84g
	SA <sub>1</sub>	18.51± 0.49de	11.82± 5.73cd	66.33± 0.88c	68.67± 11.54ef	231.26± 4.25f
	SA <sub>2</sub>	23.82± 0.33d	11.74± 7.87cd	67.66± 0.33c	106.41± 11.67a	245.74± 3.51b
I <sub>3</sub>	SA <sub>0</sub>	17.51± 0.63e	11.49± 0.60cd	59.33± 3.61de	54.17± 10.40g	216.79± 2.40i
	SA <sub>1</sub>	18.25± 0.80de	13.69± 0.17c	66.33± 3.41c	75.22± 10.32d	218.59± 2.46h
	SA <sub>2</sub>	18.76± 4.22de	11.36± 0.22cd	74.00± 0.57b	96.57± 11.17b	233.07± 2.40e
I <sub>4</sub>	SA <sub>0</sub>	6.27± 0.15f	4.15± 0.16e	57.00± 1.75e	66.28± 6.10f	147.66± 4.51l
	SA <sub>1</sub>	7.33± 0.36f	4.94± 0.18de	63.33± 1.83cd	71.24± 2.91e	149.72± 4.80k
	SA <sub>2</sub>	16.24± 0.58e	10.46± 0.20cde	74.00± 2.41b	70.80± 5.81e	160.89± 4.42g

Means with different letters in each column are significantly different at  $p \leq 0.05$  (Duncan's test); I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, I<sub>4</sub>: Irrigation after 100, 110, 130, and 160 mm evaporation, respectively; SA<sub>0</sub>, SA<sub>1</sub>, SA<sub>2</sub>: Foliar spray of water (control), and 0.6 and 1.2 mM salicylic acid

### Flower mass

The flower mass was not significantly affected under mild and moderate stresses, but it was significantly reduced due to severe water stress (Figure 1a). Foliar spray of plants by salicylic acid, especially with 0.6 mM, improved flower mass. However, there was no significant difference between untreated (SA<sub>0</sub>) and SA<sub>2</sub> treated plants (Figure 1b).

### Seed mass

Seed mass was gradually decreased with

increasing water deficit up to moderate stress and thereafter, it was considerably reduced under severe stress. The highest seed mass (255.75 g m<sup>-2</sup>) was obtained by the SA<sub>2</sub> treatment under all irrigation levels. Therefore, the lowest seed mass (147.66 g m<sup>-2</sup>) was recorded for untreated plants under severe deficit (Table 3).

The results of data analysis (Table 4) showed significant effects of irrigation and SA levels on essential oil percentage and yield of dill flowers and seeds and essential oil yield of leaves and stems. Essential oil percentage of vegetative

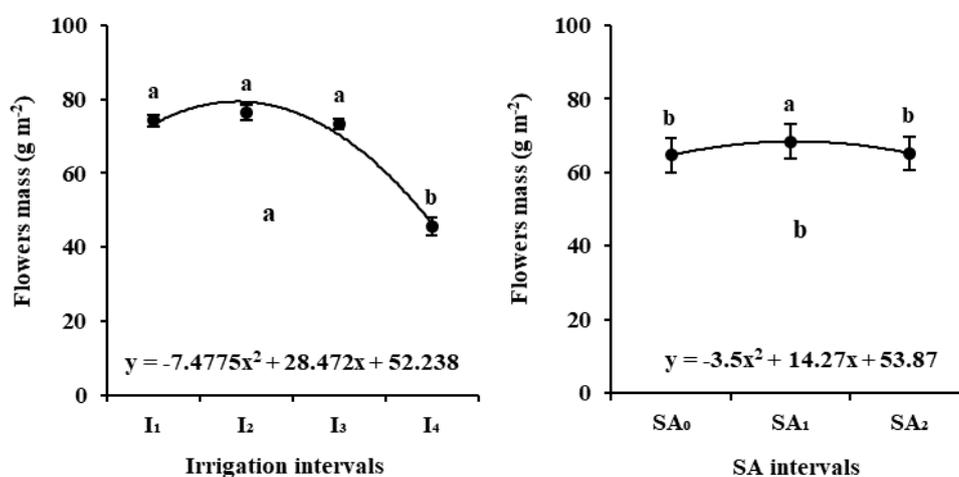


Figure 1. Variation in flower mass of dill affected by irrigation levels (a) and salicylic acid levels (b). Different letters indicate significant differences among treatments at  $p \leq 0.05$  (Duncan's test).

Table 4. The essential oil content and yield of dill organs affected by water supply and salicylic acid

Mean squares							
Treatments	df	Essential oil percentage			Essential oil yield		
		Leaves and stems	Flower	Seed	Leaves and stems	Flower	Seed
Replication	2	0.03 <sup>ns</sup>	0.07 <sup>ns</sup>	0.02 <sup>ns</sup>	22.77 <sup>ns</sup>	215.69 <sup>ns</sup>	1707.06 <sup>ns</sup>
Irrigation (I)	3	0.07*	0.44*	0.53**	368.82**	4854.46**	67086.61**
Ea	6	0.01	0.08	0.04	23.71	630.04	1382.84
Salicylic acid (SA)	2	0.00 <sup>ns</sup>	0.29**	0.48**	118.57**	2679.18**	36979.40**
I × SA	6	0.06 <sup>ns</sup>	0.04**	0.02*	14.54 <sup>ns</sup>	413.89**	3401.06 <sup>ns</sup>
Eb	16	0.01	0.09	0.09	6.60	72.49	1252.73
CV (%)	-	15.21	14.50	5.78	16.29	14.65	11.00

ns, \*, \*\*: not significant and significant at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively

organs was only affected by water stress, but not by SA. The interaction of irrigation × SA was also significant for the essential oil percentage of flowers and seeds and the essential oil yield of flowers.

### Essential oil content

The essential oil percentage increased in dill organs as a result of a decrement in the water supply. The essential oil content of leaves and stems was lower than seeds and flowers. The

essential oil production of vegetative organs increased with decreasing water supply up to moderate water deficit, but it was decreased with further increase in water limitation. However, this decline was not statistically significant (Figure 2).

The highest essential oil content of flowers (1.05%) was obtained under moderate water deficit (I<sub>3</sub>) with SA<sub>2</sub> treatment. However, there was no significant difference between 0.6 mM and 1.2 mM salicylic acid under this level of stress. The lowest essential oil percentage was recorded

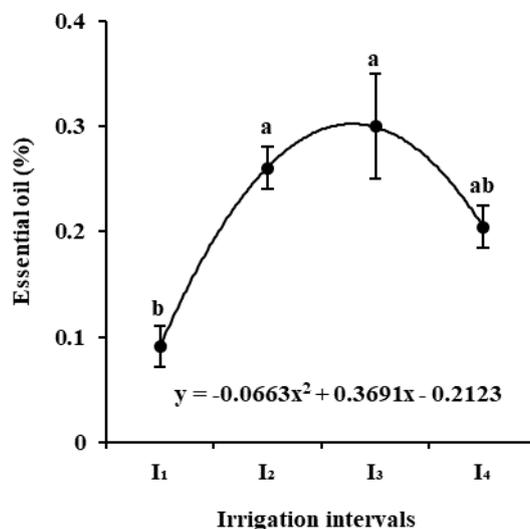


Figure 2. Variation of essential oil percentage in vegetative organs of dill in response to irrigation levels. Different letters indicate significant differences among treatments at  $p \leq 0.05$  (Duncan's test).

in the SA-untreated plants under normal irrigation. The maximum essential oil content of seeds (1.97%) was recorded for the SA<sub>2</sub>-treated plants under moderate water deficit, although there was no significant difference between mild and moderate stresses in these plants (Figure 3).

The essential oil yield of leaves and stems was significantly enhanced under moderate and severe water stress, compared with normal irrigation. However, there was no significant difference among mild, moderate, and severe stresses. The highest improvement in essential oil yield was achieved by the SA<sub>2</sub> treatment, followed by SA<sub>1</sub>. The maximum essential oil yield of seeds was produced under mild water deficit, which had no significant difference with moderate water deficit. Foliar spray of SA<sub>2</sub> was again the superior treatment for enhancing the essential oil yield of dill plants (Table 5).

The essential oil yield of flowers was

enhanced by the SA treatment, especially under severe water limitations. This augmentation of essential oil production by SA spray was not significant under normal irrigation but considerably increased under limited water supply, particularly under moderate stress. The most effective treatment for improving the essential oil yield of dill flowers under all irrigation levels was the SA<sub>2</sub> spray on plants (Figure 4).

## Discussion

Chlorophyll as a major pigment of plants has a positive relationship with photosynthetic rate. Reduction in chlorophyll content of dill leaves due to water deficit (Table 3) might be related to the enhanced activity of the chlorophyllase, which degrades the chloroplast structure (Singh *et al.* 2010). The decrement of chlorophyll in drought-stressed plants was also the consequence of

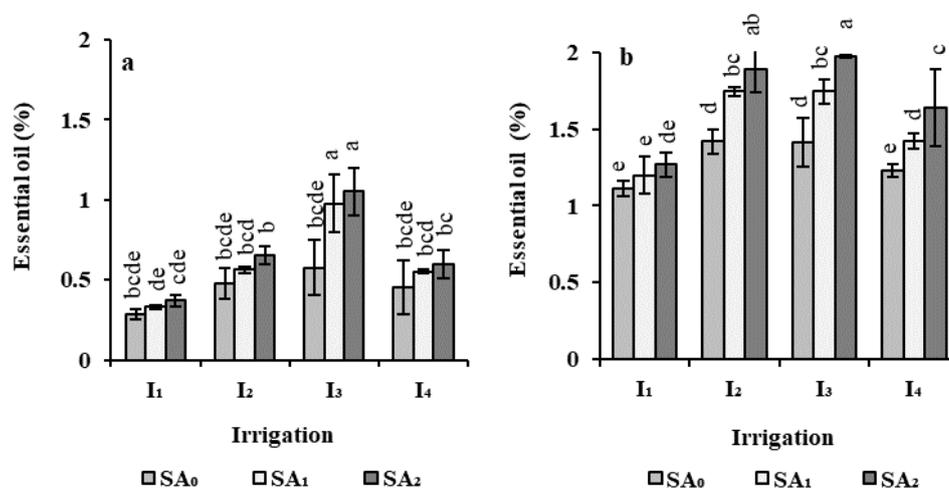


Figure 3. Means comparison essential oil percentage of dill flowers (a) and seeds (b) affected by irrigation and SA levels. Different letters indicate significant differences among treatments at  $p \leq 0.05$  (Duncan's test).

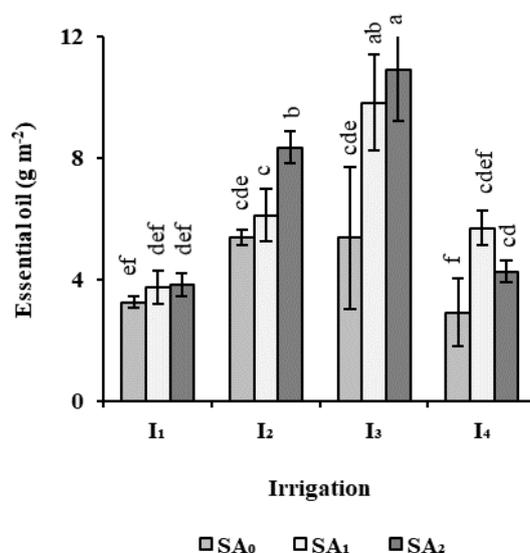


Figure 4. The essential oil yield of dill flowers for irrigation  $\times$  SA interaction; Different letters indicate significant differences among treatments at  $p \leq 0.05$  (Duncan's test).

Table 5. The essential oil yield of dill organs for different irrigation and salicylic acid levels

Treatments	Essential oil yield ( $\text{g m}^{-2}$ )	
	Leaves and stems	Seed
Irrigation (I)		
I <sub>1</sub>	7.33 $\pm$ 0.85b	29.98 $\pm$ 1.46b
I <sub>2</sub>	15.10 $\pm$ 2.42ab	40.80 $\pm$ 3.28a
I <sub>3</sub>	22.53 $\pm$ 2.80a	36.85 $\pm$ 1.53a
I <sub>4</sub>	18.13 $\pm$ 2.86a	21.10 $\pm$ 1.85c
Salicylic acid (SA)		
SA <sub>0</sub>	12.69 $\pm$ 3.24b	26.76 $\pm$ 2.97c
SA <sub>1</sub>	15.66 $\pm$ 2.79b	31.93 $\pm$ 2.77b
SA <sub>2</sub>	18.98 $\pm$ 2.44a	37.86 $\pm$ 2.96a

Means with different letters in each column are significantly different at  $p \leq 0.05$  (Duncan's test).

oxidative stress that may have resulted in photo-oxidation and degradation of this pigment (Anjum *et al.* 2011). Some researchers reported that decreasing carbon efficiency and increasing ethanol and lactate production under stress can reduce the synthesis of photosynthetic pigments (Sayyad-Amin *et al.* 2016). The augmentation of chlorophyll content with 1.2 mM SA spray (Table 3) may be associated with enhancing nitrogen absorption and nitrate reductase activity (Singh and Usha 2003; Afroz *et al.* 2006). Nitrogen is the most important component of chlorophyll. Therefore, low nitrogen availability can reduce chlorophyll content and consequently the photosynthesis rate (Toth *et al.* 2002). Exogenous SA can considerably reduce chlorophyll degradation via increasing antioxidant enzymes activities and thereby scavenging reactive oxygen species (Farhangi-Abriz and Ghassemi-Golezani 2018).

The low green cover of water-stressed dill plants (Table 3) was the consequence of plants' competition for water and nutrients (Dhima *et al.* 2009). The decrease in green cover was associated with a decrease in chlorophyll content under water stress as it reduced photosynthesis and subsequent growth (Table 3). Drought decreases cell turgor and plant growth, leading to a reduction in leaf area (Zlatev and Cebola Lidon 2012) and ground green cover (Zhang *et al.* 2020). The application of SA increases the number of leaves (Zamaninejad *et al.* 2013) and chlorophyll content (Table 3) under stressful conditions. These positive effects of SA may also be related to its

crosstalk with other phytohormones. The SA alters the auxin, cytokinin, and ABA levels and improves the growth and productivity of plants under normal and stressful conditions (Shakirova 2007).

Significant reduction of plant organ masses (leaves, stems, flowers, and seeds) under severe water deficit (Table 3; Figure 4a) could be related to a decline in chlorophyll content (Table 3) and ground green cover (Table 3). According to Zafar *et al.* (2019), decreasing the dry mass of plants under drought stress was associated with leaf senescence and a decrement in cell enlargement resulting from reduced turgor pressure. Drought stress also limits the photo-assimilation metabolites required for cell division. Water stress can likely reduce the metabolic activity of plant cells, which in turn may decrease their growth and productivity (Okunloia *et al.* 2017). The consequence of these impairments is the reduction of plant growth and biomass. However, slight effects of water limitation up to moderate stress (Table 3; Figure 4a) indicate that dill plants can somewhat tolerate water shortages.

Enhancing organs masses of the SA-treated plants, especially with 1.2 mM (Table 3; Figure 4b) were related to higher chlorophyll content (Table 3) and ground cover of these plants (Table 3), which improved photosynthetic activity and photo-assimilate availability for different organs (Osorio *et al.* 2014). Ground green cover is highly correlated with the light interception (Evangelos *et al.* 2012). Therefore, the higher values of this trait can likely enhance crop growth and yield.

Rising light trapping by the well-expanded green canopy cover (Table 3) with higher chlorophyll content (Table 3) can improve assimilate synthesis during plant growth and development, thereby increasing dill organs masses (Table 3; Figure 4b).

Increasing essential oil percentage and yield of vegetative organs, flowers, and seeds (Figures 2, 3, 4) with increasing water deficit up to moderate stress is a defense mechanism against environmental stresses (Tasiu-Isah 2019). In other words, the stress conditions stimulate the biosynthesis of essential oil (Ezz *et al.* 2009). The rising of essential oil content of medicinal plants under drought stress is a way of inhibiting oxidization within the plant cells (Aliabadi *et al.* 2009). According to Delfine *et al.* (2005) reduction of the plant leaf area due to water stress can enhance the density of the glandular trichomes which increases essential oil accumulation per unit leaf area. Production and accumulation of essential oil in leaf tissues can protect plant cells from possible damages of ROS under stressful conditions (Ramakrishna and Ravishankar 2011). A low allocation of carbon to the growth under drought stress is a choice between growth and defense for the plants (Turtola *et al.* 2003). Environmental stresses such as water shortage increase the biosynthesis rate of essential oil through a passive shift in their redox potential. In addition, the status of the enzymes involved in the biosynthesis of essential oil could be up-regulated when subjected to adverse environmental conditions (Selmar *et al.* 2017). Decreasing essential oil production of vegetative organs, flowers, and seeds due to severe stress

(Figures 2, 3) might be the result of a reduction in glandular trichomes (Yadav *et al.* 2014). A large reduction in plant organs masses (Table 3; Figure 4a) under severe water limitation was also led to a decline in essential oil yield of different organs (Figure 4).

Improvement of essential oil percentage and yield of dill organs by foliar application of SA (Figures 3, 4; Table 5) might be resulted from the regulating effects of this hormone on plant growth and development. Salicylic acid may change secondary metabolites through enhancing chlorophyll level (Nourafcan *et al.* 2014), photosynthesis, transpiration, and stomatal conductance (Stevens *et al.* 2006). Also, according to Rowshan *et al.* (2010), the exogenous SA was effective in promoting the production of secondary metabolites in the plant cell and tissue culture. SA may also induce the expression of many defence genes which involve in activation of the enzymes responsible for secondary metabolite production (Khan MIR *et al.* 2015), which can protect plants subjected to abiotic stress conditions (Ali *et al.* 2007). The superiorities of the SA-treated plants in essential oil production were related to enhanced growth and biomass of different organs (Yeganehpour *et al.* 2016).

Reduction in essential oil yield of seeds under severe water stress is strongly associated with the reduction of seed mass under stress (Table 3), which is also supported by a previous report (Ghassemi-Golezani *et al.* 2008). Nevertheless, enhancing essential oil yield of foliar-treated and untreated plants under stressful

conditions, especially under mild and moderate stresses (Table 5; Figure 4) suggests that water stress is not always completely detrimental (Osakabel *et al.* 2014; Rosa *et al.* 2019).

### Conclusions

The high essential oil yield of dill organs under mild and moderate water stress was the result of great enhancement in essential oil content (Figures 3, 4), with no significant loss of organs masses (Table 3; Figure 4). Enhancing chlorophyll content (Table 3), green cover (Table 3), organs masses (Table 3; Figure 4), and essential oil content of flowers and seeds (Figure 3) by the SA treatment, particularly by 1.2 mM SA, was led to a considerable improvement in

essential oil yield under limited irrigation conditions. Therefore, foliar spray of 1.2 mM SA with limited irrigation of plants were the most beneficial treatments for essential oil production of dill organs, with the superiority of seeds followed by flowers and vegetative organs.

### Acknowledgement

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### Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

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## تغییرات رشد و محتوای اسانس اندام های شوید (*Anethum graveolens*) تحت تنش خشکی در واکنش به سالیسیلیک اسید

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### چکیده

برخی از اثرات مضر محدودیت آب بر عملکرد گیاه زراعی ممکن است توسط تنظیم‌کننده‌های رشد مانند سالیسیلیک اسید کاهش یابد. بنابراین، آزمایشی به صورت کرت‌های خردشده در قالب طرح بلوک‌های کامل تصادفی در سه تکرار برای ارزیابی تغییرات محتوای اسانس اندام‌های شوید (*Anethum graveolens* L.) در واکنش به فراهمی آب (تأمین آب پس از ۷۰، ۱۰۰، ۱۳۰ و ۱۶۰ میلی‌متر تبخیر به ترتیب به عنوان آبیاری معمول و تنش‌های ملایم، متوسط و شدید) و سطوح سالیسیلیک اسید (آب‌پاشی و ۰/۶، ۱/۲ میلی مولار سالیسیلیک اسید) ترتیب داده شد. فواصل آبیاری و تیمارهای سالیسیلیک اسید به ترتیب به کرت‌های اصلی و فرعی اختصاص داده شدند. نتایج نشان داد که کلروفیل a و به ویژه کلروفیل b با افزایش تنش خشکی کاهش یافته‌اند. پوشش سبز زمین و وزن اندام‌های گیاه (شاخه و برگ، گل‌ها و بذرها) فقط تحت کمبود شدید آب کاهش یافتند. درصد اسانس اندام‌های شوید با افزایش کمبود آب تا تنش متوسط فزونی یافت، اما پس از آن با تشدید کمبود آب کم شد. بیشترین محصول اسانس بخش‌های رویشی و گل‌ها نیز در گیاهان با تنش متوسط تولید شد، اما بیشترین محصول اسانس بذرها تحت کمبود ملایم آب ثبت گردید. کاربرد خارجی سالیسیلیک اسید درصد اسانس همه اندام‌های شوید را، به ویژه تحت محدودیت متوسط آب، افزایش داد. محصول اسانس اندام های شوید نیز با تیمار سالیسیلیک اسید افزایش یافت. کاربرد ۱/۲ میلی مولار سالیسیلیک اسید در بهبود تولید اسانس شوید مؤثرتر بود.

واژه‌های کلیدی: کلروفیل؛ شوید؛ اسانس؛ پوشش سبز؛ سالیسیلیک اسید؛ تنش آبی