IMPROVING RESISTANCE OF *FOENICULUM VULGARE* TO WATER DEFICIT STRESS BY NATURAL REGULATORS

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Sorkhi, F., Rostami, R. and Ghassemi-Golezani, K. (2021). Improving resistance of *Foeniculum vulgare* to water deficit stress by natural regulators. *Agriculture (Pol'nohospodárstvo)*, 67(1), 29–41.

This research was conducted as a combined analysis with four replications in two years (2018–2019). Treatments were irrigation up to 90%, 50%, and 20% field capacity (as normal irrigation, moderate and severe water deficit stresses, respectively) and foliar application of natural regulators (untreated as control, salicylic acid, spermidine, and methanol). Increasing water deficit stress was led to a significant increase in essential oil percentage and proline content and a significant decrease in yield parameters and seed yield. Most of the traits (except the percentage of essential oil) were affected by natural growth regulators. The highest seed yield (1,127.59 kg/ha), plant biomass (5,426.92 kg/ha), essential oil yield (22.67 kg/ha), and proline content (29.34 µmol/g fresh weight) were obtained in methanol treated plants under normal irrigation. However, the highest amount of these traits under moderate and severe water deficit was recorded for salicylic acid-treated plants. Therefore, foliar spray of methanol was a useful treatment for non-stress conditions, but, application of salicylic acid was the superior treatment for reducing the negative effects of water deficit stress on *Foeniculum vulgare*.

Key words: essential oil, fennel, salicylic acid, stress, yield

Fennel (*Foeniculum vulgare*) is one of the most important medicinal plants of the Apiaceae family (Ibrahim *et al.* 2020). Fennel essential oil is used in many industries such as pharmaceutical, food, cosmetic, and health (Pouryoucef 2014). The amount of essential oil produced in medicinal plants is affected by environmental stresses such as water deficit (Tatrai *et al.* 2016). Water deficit causes the production of reactive oxygen species (ROS) in plants and consequently the reactive oxygen species damages biomolecules such as lipids, proteins, and nucleic acids (Tardieu *et al.* 2018). Some of the detrimental impacts of water shortage on plants may be alleviated by foliar application of natural products such as

salicylic acid, spermidine, and methanol (Hossinza-deh *et al.* 2015; Ghilavizadeh *et al.* 2019).

Salicylic acid or ortho-hydroxybenzoic acid belongs to the group of phenolic compounds (Mabrouk *et al.* 2019), that induce drought tolerance in plants (Roghayyeh *et al.* 2014), and is considered as a plant hormone (Seagate *et al.* 2020). In the study of seed pre-treatment with salicylic acid, the negative effects of water deficit stress on seed yield, yield components, and the amount of fennel essential oil were reduced due to the application of this growth regulator in stress-free conditions, but it had no significant effect on seed yield (Rizwan *et al.* 2020). Najmeh *et al.* (2015) stated that foliar application

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of salicylic acid under drought stress increased the yield of *Nigella sativa* by 79.05% and 10 μ M salicylic acid under moderate stress increased the essential oil content by 150%.

Antioxidant and anti-ethylene compounds help plants to maintain membranes and their physiological function under drought stress (Li *et al.* 2015). Polyamines, including spermidine, inhibit ethylene production (Kang *et al.* 2013). The results of Shakib *et al.* (2013) showed the beneficial effects of foliar application of spermidine on flowering and vegetative characteristics of *Cyclamen persicum*. The application of spermidine in the concentration 0.2 mM improved the flowering period, flower life, and increased the number of flowers, while the 0.4 mM concentration improved some vegetative characteristics such as the number of leaves. Niakan *et al.* (2011) reported that foliar application of spermidine under salt stress significantly decreased the inhibitory effects of salinity on germination percentage, root length, shoot length, fresh weights, and dry weight, and also the content of osmotic regulators in wheat seedlings. Some reports have suggested that salicylic acid plays a much more successful role than spermidine in controlling the effects of water deficit (Rizwan *et al.* 2020; Seagate *et al.* 2020). Some studies have shown that methanol application on most crops decreases photorespiration, and increases water use efficiency, leaf area, and ultimately yield (Dorokhov *et al.* 2015). Research on the effect of foliar application of methanol under water deficit stress revealed that biomass of plants increases this

treatment (Mirakhori *et al.* 2009; Moghadas *et al.* 2013). According to research such as salicylic acid in *Triticum aestivum* (Seagate *et al.* 2020), *Nigella sativa* (Najmeh *et al.* 2015), and *Foeniculum vulgare* (Ghilavizadeh *et al.* 2019), methanol in *Cicer arietinum* (Hossinzadeh *et al.* 2015) and spermidine in *Cuminum cyminum* (Bakhtati *et al.* 2016) growth regulators cause resistance to drought stress and significantly increase crop growth and yield. Iran is located in an arid and semi-arid region, where prevention or reduction of damages on plants due to water deficit is very important. So, this research was conducted to evaluate the effects of salicylic acid, methanol, and spermidine on *Foeniculum vulgare* under different levels of water supply.

MATERIAL AND METHODS

This research was conducted as a combined analysis with four replications in two years (2018–2019) in the Research Farm of Miandoab Branch of Azad University, Iran. The climate of Iran is arid and semi-arid. The geographical coordinates of Miandoab city include longitude with 48 degrees and 52 minutes east and latitude with 35 degrees and 20 minutes north. The altitude of the region is 1,300 meters above sea level. The climatic conditions of the region during the experiment at the study site are presented in Table 1. Treatments were irrigation up to 90%, 50%, and 20% field capacity (as normal irrigation, moderate and severe stresses, respectively)

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Climate of the region during the experiment

Month	2018				Month	2019			
	Minimum temperature [°C]	Maximum temperature [°C]	Rainfall [Mm]	Relative humidity [%]		Minimum temperature [°C]	Maximum temperature [°C]	Rainfall [Mm]	Relative humidity [%]
April	3.6	13.1	25.9	49	April	4.0	18.4	24.7	46
May	8.1	18.7	26.6	47	May	8.9	24.5	37.8	52
June	11.4	25.6	20.6	42	June	12.7	30.8	29.7	46
July	15.3	28.1	16.7	41	July	16.6	34.4	12.7	37
August	16.8	29.7	7.9	33	August	18.4	36.8	4.2	30
September	14.3	26.7	11.4	36	September	16.7	33.8	9.6	32

and foliar application of natural regulators included untreated as control, salicylic acid at a concentration of 1 mM (Chen *et al.* 2016), spermidine with the concentration of 0.2 mM (Li *et al.* 2015) and methanol with the concentration of 30% by volume (Zheng *et al.* 2008). To determine the soil moisture and irrigation time in each test plot, a tensiometer model 2710ARL made in the USA was installed in the depth of 15 cm. Irrigation was performed whenever the humidity reached the desired level for irrigation (in the present study, the amount of soil water potential with –0.3 bar equivalent to field capacity, –1.47 bar equivalent to 90% of field capacity, –7.35 bar equivalent to 50% of field capacity, –11.76 bar equivalent to 20% of field capacity, and –15 bar equivalent to permanent wilting of the plant). The physical and chemical characteristics of the soil at the study site are presented in Table 2.

The physical and chemical properties of soil were determined by conventional laboratory methods. In order to measure soil samples pH, in suspension with a ratio of 2 to 1 solution to soil was used (Thomas 1996). Electrical conductivity was measured in filtered extracts with a ratio of 2 to 1 solution to soil (Rhoades 1996). By titration recurrence method, soil calcium carbonate was measured with one normal hydrochloric acid (Loeppert & Sparks 1996). By wet oxidation method, the percentage of soil organic carbon was measured (Nelson & Sommers 1996). By the hydrometric method, soil texture was measured (Gee & Bauder 1986). By digestion method with nitric acid and perchloric acid, soil phosphorus was measured too (Kuo 1996).

In both years after plowing the land in March, the land was levelled and plotted. The size of each plot was 3×4 square meters. In each plot, 6 planting rows were created with a distance of 50 cm from each other. Fennel seeds were planted 15 cm apart

on rows with a depth of 2 to 3 cm. Planting was done in April and irrigation was done immediately after planting. When the plants reached a height of 5 cm, the field was thinned to obtain the proper density.

Proline content

The amount of plant proline in the 75% flowering stage was measured by Bates *et al.* (1973) method and was reported based on micromol of proline per gram of fresh leaf (μmol/g FW).

Yield and yield components

After harvesting in the second half of September, 7 plants were randomly selected and then measured plant height, number of branches per plant, number of umbels per plant, number of seeds per umbel, and weight of one thousand seeds. To determine the yield in each plot, two side rows and a half meter from the first and a half meter from the end of the plot were removed, biological yield and seed yield were determined at the level of 6 square meters.

Harvest index

The following equation was used to calculate the harvest index (Ntanos & Koutroubas 2002).

$$HI = (SY/BY) \times 100$$

HI – Harvest index (percentage); SY – Seed yield [kg/ha] and BY – Biological yield [kg/ha].

Essential oil

The amount of 50 g of seeds produced in each plot was randomly selected. First, the seeds were thoroughly ground, then poured into a 1-liter balloon and 750 ml of water was added to it. Then, the solution was placed in a cleverger apparatus for 4 hours. After dehumidification, essential oil percent was determined by sodium sulfate using the Telci method (Telci *et al.* 2009). Essential oil yield was

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Physical and chemical properties of soil

Soil Texture	Silt [%]	Clay [%]	Sand [%]	Total N [%]	K [mg/kg]	P [mg/kg]	OC [%]	EC [dS/m]	pH
Silty loam	57.7	21.8	20.5	0.08	264.17	20.25	0.83	1.29	7.19

OC – organic carbon; EC – electrical conductivity of soil solution

calculated by multiplying seed yield by the percentage of essential oil (Stefanini *et al.* 2006).

SAS software was used for statistical analysis of data and Excel software was used to draw graphs. The means were compared with Duncan's multiple range test at a 5% probability level.

RESULTS AND DISCUSSION

Plant height

Plant height at the level of one percent probability was affected by the simple effects of water deficit stress and natural regulators, but the interaction of these two treatments had no significant effect on plant height (Table 3). The highest plant height of the fennel was observed non-water deficit stress treatment (irrigation 90% of field capacity) with 86.14 cm.

Under the influence of moderate and severe water deficit stresses, plant height decreased by 25.04% (64.57 cm) and 45.17% (47.23 cm) compared to the non-stress treatment (86.14 cm), respectively (Table 4). The first noticeable effect of water deficit stress on the plant is to reduce plant height (Sah *et al.* 2014). Plant height is greatly affected by genetic characteristics (Ahmadizadeh *et al.* 2012). However, environmental conditions such as water deficit stress significantly affect plant height (Tatrai *et al.* 2016). According to research results, water shortage reduces the turgor pressure of stomatal guard cells, stomatal conduction, growth rate, photosynthesis rate and as a result, morphological characteristics are reduced such as plant height (Najmeh *et al.* 2015; Liu *et al.* 2017). Plant growth is associated with an increase in cell size and this is the most sensitive process in plants to water deficit stress (Seagate *et al.* 2020). In conditions without water stress, increasing growth and leaf area has a direct effect on increasing the absorption of solar energy and leads to an increase in plant height (Shakib *et al.* 2013). The reduction of water during plant growth and development has a negative effect on plant growth parameters (Rizwan *et al.* 2020). Askari and Ehsanzadeh (2015) reported that irrigation at the time of the evacuation of 75% and 85% of soil moisture, led to a reduction of 20% and 31% in height of fennel plant compared to irrigation at the time of the evac-

uation 35% of soil moisture. Kouchaki *et al.* (2006) showed that with increasing the irrigation period from 10 to 20 and 30 days, the height of the fennel plant decreased significantly. It has been found that drought stress reduces plant height by reducing plant growth rate (Liu *et al.* 2017). Foliar application of methanol and salicylic acid significantly increased plant height compared to control and spermidine. This increase in plant height was 11.67% (68.02 cm) with methanol foliar application and 22.06% (74.35 cm) with salicylic acid foliar application compared to the control treatment (60.91 cm) (Table 4). About 90% of the dry weight of plants is due to the assimilation of CO₂ by photosynthesis and increasing the rate of photosynthesis is useful to increase the production capacity of crops (Tatrai *et al.* 2016). Plants can easily absorb methanol foliar application and use it as a carbon source (Tardieu *et al.* 2018). Salehi (2013) confirmed that methanol foliar application significantly increased bean plant height. Ahmadian *et al.* (2011) showed that spermidine increased the height of *Matricaria chamomilla*, but this increase was statistically lower compared to salicylic acid.

Salarpour and Frahbakhash (2014) reported that increasing the levels of salicylic acid from zero to one mM, significantly increased the height of the fennel plant. They stated that salicylic acid increases cell division in the plant meristem and thus increases plant height. The results of another study showed that salicylic acid improves photosynthesis and plant growth by increasing the activity of the rubisco enzyme (Seagate *et al.* 2020). Salicylic acid seems to regulate various physiological processes such as plant growth and development through the synthesis of special proteins called protein kinases, which are responsible for regulating cell division, differentiation, morphogenesis and play an effective role in increasing plant height.

Yield components

All traits related to yield components including the number of branches per plant, number of umbels per plant, number of seeds per umbel, and weight of one thousand seeds were significantly affected by the interaction water deficit stress, and natural regulators (Table 3).

Investigation of the interaction effects of water deficit stress with natural regulators showed that

under control treatment (irrigation at 90% of field capacity) compared to moderate and severe water deficit stress treatments in methanol foliar application had the highest number of branches and with increasing water deficit stress, the amount of this trait in all foliar applications decreased significantly. The results showed that in the lack of water deficit stress, methanol foliar application had the highest number

of branches (15.28) and increased the amount of this trait in the fennel by 8.44% compared to the control (non-foliar application) treatment with 14.09 number (Table 5).

The use of salicylic acid in moderate water deficit stress (50% of field capacity) increased the number of branches by 47.08% (12.09) compared to control (non-foliar application) with 8.22 num-

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Analysis of variance of evaluated traits in *Foeniculum vulgare*

S.O.V	df	Plant height	Number of branches per plant	Number of umbels per plant	Number of seeds per umbel	Weight of one thousand seeds	Biological yield
Year	1	9.24	5.34	13.86	2.28	1.06	114.60
Year×Replication	6	29.60	7.82	10.45	21.70	3.11	125.21
Water deficit stress (WDS)	2	183.75**	16.51*	23.79*	68.47**	13.70**	661.74**
Year×WDS	2	13.99	2.93	6.22	9.16	3.52	70.59
Natural regulators (NR)	3	192.67**	14.04*	17.34*	88.42**	19.07**	397.35*
Year×NR	3	10.86	1.38	7.92	3.04	4.18	68.39
WDS×NR	6	3.50	11.97*	34.80**	29.94*	16.25**	278.46*
Year×WSD×NR	6	12.32	5.81	6.56	7.53	2.78	75.06
Error	66	15.95	4.37	5.02	11.93	2.27	106.53
CV		11.38	6.52	9.13	7.95	2.63	12.71

Table 3 – continue

S.O.V	df	Seed yield	Harvest index	Essential oil percent	Essential oil yield	Proline content
Year	1	95.32	5.83	0.28	0.76	4.25
Year×Replication	6	93.17	7.95	0.17	0.82	9.21
Water deficit stress (WDS)	2	276.51**	16.27*	0.83**	11.49*	25.47*
Year×WDS	2	26.33	4.58	0.07	2.80	9.17
Natural regulators (NR)	3	250.24**	15.91*	0.23	8.75*	20.93*
Year×NR	3	54.32	2.02	0.04	3.14	8.33
WDS×NR	6	315.17**	5.34	0.03	7.39*	23.44**
Year×WSD×NR	6	14.49	1.68	0.11	2.69	7.75
Error	66	48.78	4.10	0.13	2.91	6.47
CV		9.23	6.36	2.72	5.14	8.96

* and ** – significant difference $P \leq 0.05$ and significant difference $P \leq 0.01$, respectively (S.O.V: Sources of variation)

Note: df – degrees of freedom; CV – coefficient of variation

ber and in severe water deficit stress (25% of field capacity) increased number of branches by 90.45% (8.18) compared to control (non-foliar application) with 4.61 number (Table 5). The highest number of umbels per plant (29.67) was observed in non-stress conditions in methanol foliar application which was 18.53% higher than the control (non-foliar application) treatment. In moderate and severe stress treatments, salicylic acid was able to increase the number of umbels per plant compared to the control treatment by 21.96% (23.04 number) and 73.14% (17.54 number), respectively (Table 5). With increasing water deficit stress, the number of seeds per umbel decreased in all foliar application treatments. The highest number of seeds per umbel with 245.60 was observed to methanol foliar application in non-stress conditions (irrigation at 90% of field capacity) and the lowest belonged to control treatment in severe stress (irrigation at 20% of field capacity). In moderate water deficit stress, foliar application with salicylic acid increased the number of seeds per umbel by 48.86% (186.31 number) compared to control (125.15 number). In severe water deficit stress, foliar application with salicylic acid increased the number of seeds per umbel by 75.62% (135.51 number) compared to control (77.16 number). The results of this study confirmed that in non-stress conditions, foliar application with methanol and in stress conditions, foliar application with salicylic acid were the most effective treatments. In non-stress treatment

(irrigation at 90% of field capacity), no significant difference was observed between the weight of one thousand seeds in foliar application treatments (Table 4). Salicylic acid application in moderate stress (irrigation at 50% of field capacity) increased the weight of one thousand seeds by 22.10% (5.91 g) compared to the control (non-foliar application) with 4.84 g and use of salicylic acid in severe stress treatments (irrigation at 20% of field capacity) increased weight of one thousand seeds by 52.64% (4.61 g) compared to the control (3.02 g). However, methanol treatment significantly increased the weight of one thousand seeds in water deficit stress conditions compared to the control, which was not statistically significant with salicylic acid treatment (Table 5).

The reduction of fennel yield components under water-deficit stress conditions has been confirmed by various researchers (Kouchaki *et al.* 2006; Askari & Ehsanzadeh 2015). The main reason for the decrease in the yield components of the fennel due to water deficit stress related to the disturbance of pollination as a result of water shortage during the flowering stage and sterilization of emerging umbels. It seems that the role of methanol is more nutritional (Zbiec *et al.* 2003; Moghadas *et al.* 2013) and salicylic acid has an effective role in fennel resistance to water deficit stress (Kabiri *et al.* 2014). Methanol has an effect on increasing growth by increasing photosynthetic activity, increasing cytokine production and

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Effect of water deficit stress and natural regulators on plant height, harvest index and essential oil percent of *Foeniculum vulgare*

Treatment		Plant height [cm]	Harvest index [%]	Essential oil [%]
Water deficit stress	90%	86.14 ± 5.75 ^a	18.90 ± 1.12 ^a	2.56 ± 0.05 ^c
	50%	64.57 ± 3.92 ^b	19.51 ± 1.67 ^a	3.13 ± 0.07 ^b
	20%	47.23 ± 4.84 ^c	13.24 ± 0.95 ^b	3.98 ± 0.18 ^a
Natural regulators	Control	60.91 ± 3.68 ^c	16.12 ± 0.26 ^b	3.18 ± 0.1 ^a
	Spermidine	62.88 ± 3.04 ^c	16.35 ± 0.95 ^b	3.22 ± 0.19 ^a
	Salicylic acid	74.35 ± 4.11 ^a	18.73 ± 1.06 ^a	3.31 ± 0.08 ^a
	Methanol	68.02 ± 2.95 ^b	17.99 ± 0.52 ^a	3.29 ± 0.12 ^a

Means in each column with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

stimulating plant growth, increasing nitrate reductase activity, increasing nitrogen metabolism, and decreasing light respiration, thus improving the fennel yield components (Zbiec *et al.* 2003; Dorokhov *et al.* 2015). The reason for the decrease in light respiration in methanol-treated plants is the rapid oxidation of methanol to carbon dioxide and its combination with ribulose 1–5 bisphosphate carboxylase and reduced oxygen competition (Hossinzadeh *et al.* 2015). Yield components of cumin increased significantly under drought stress with methanol application but not significantly increased with spermidine application (Nejhad & Moghaddam 2010). Ramirez *et al.* (2006) reported that the treatment of tobacco leaves with methanol significantly increased the chlorophyll content of the leaves of this plant. The positive effect of methanol on the growth of various plants under water deficit stress conditions has been confirmed by many researchers (Mirakhori *et al.* 2009; Moghadas *et al.* 2013).

Biological yield

The interaction of water deficit stress and natural regulators significantly affected ($P \leq 0.05$) the

biological yield of the fennel (Table 3). Water deficit stress reduces the turgor pressure, reduces cell growth and plant organ growth (Tardieu *et al.* 2018). On the other hand, water deficit stress reduces characteristics such as water uptake, nutrients, leaf area, plant growth rate, plant growth period, and these factors ultimately lead to reduced dry matter production (Sah *et al.* 2014). There was no significant difference between different natural regulators treatments in biological yield in non-water stress treatment (irrigation at 90% of field capacity). The highest biological yield of the plant (5,426.92 kg/ha) was observed in methanol foliar application under non-stress conditions. Salicylic acid treatment at moderate water deficit stress (4,662.54 kg/ha) and severe water deficit stress (3,479.02 kg/ha) produced the highest biological yield, which no significant difference between foliar application of salicylic acid and methanol under water deficit stress. In moderate and severe stress treatment, foliar application with salicylic acid increased biological yield by 30.27% and 77.34%, compared to the control treatment, respectively (Figure 1). Methanol increased some of the agronomic characteristics of sugar beet such as

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Interaction of water deficit stress and natural regulators on number of branches per plant, number of umbels per plant, number of seeds per umbel, and weight of one thousand seeds of *Foeniculum vulgare*

Treatment		Number of branches per plant	Number of umbels per plant	Number of seeds per umbel	Weight of one thousand seeds [g]
Water deficit stress	Natural regulators				
90%	Control	14.09±0.26 ^b	25.03±0.76 ^b	223.95±8.46 ^a	5.59±0.077 ^{ab}
	Spermidine	13.92±0.34 ^b	25.79±0.84 ^b	224.07±7.65 ^a	5.61±0.073 ^{ab}
	Salicylic acid	13.78±0.51 ^b	26.50±0.51 ^b	224.89±8.02 ^a	5.85±0.084 ^a
	Methanol	15.28±0.56 ^a	29.67±1.06 ^a	245.60±7.91 ^a	5.93±0.095 ^a
50%	Control	8.22±0.88 ^f	18.89±0.62 ^e	125.15±6.38 ^c	4.84±0.054 ^c
	Spermidine	10.21±0.17 ^e	19.42±0.09 ^e	124.21±5.29 ^c	5.03±0.065 ^b
	Salicylic acid	12.09±0.13 ^c	23.04±0.77 ^c	186.31±6.25 ^b	5.91±0.072 ^a
	Methanol	11.05±0.09 ^d	20.52±0.43 ^d	166.54±5.34 ^b	5.78±0.061 ^a
20%	Control	4.61±0.24 ^h	10.13±0.25 ^h	77.16±4.19 ^d	3.02±0.035 ^d
	Spermidine	4.59±0.31 ^h	10.16±0.18 ^h	75.28±4.41 ^d	3.35±0.028 ^d
	Salicylic acid	8.18±0.72 ^f	17.54±0.34 ^f	135.51±6.43 ^c	4.61±0.047 ^c
	Methanol	5.29±0.15 ^g	12.29±0.26 ^g	94.10±3.72 ^d	4.48±0.051 ^c

Means in each column with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

Biological yield (Nadali *et al.* 2010). Zbeik *et al.* (2003) reported that bean, rapeseed, tomato, and sugar beet treated with methanol were less sensitive to water deficiency and produced the same yield as control plants. Salicylic acid improves plant root growth by improving plant photosynthesis under water deficit stress conditions and this increase in the growth of the root system by salicylic acid causes more absorption of water and nutrients, which ultimately leads to an increase in

plant biomass (Najmeh *et al.* 2015; Rizwan *et al.* 2020).

Seed yield

The interaction of water deficit stress and natural regulators significantly affected ($P \leq 0.01$) the seed yield of the fennel (Table 3). In water deficit stress treatments, seed yield in all-natural regulators treatments decreased significantly (Figure 2). Mehta *et al.* (2011) reported that the seed yield of fennel

Figure 1. Interaction of water deficit stress and natural regulators on biological yield of *Foeniculum vulgare*
Note: Means with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

Figure 2. Interaction of water deficit stress and natural regulators on seed yield of *Foeniculum vulgare*
Note: Means with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

at irrigation levels at 75% and 50% of field capacity compared to full irrigation decreased by 22.3% and 51%, respectively. They stated that water deficit stress levels, plant growth parameters decreased and the combination of these factors eventually led to a decrease in fennel seed yield. The use of methanol foliar application treatment under non-water deficit stress conditions and salicylic acid treatment under stress condition significantly improve seed yield (Figure 2).

The highest seed yield (1,127.59 kg/ha) belonged to methanol foliar application in the non-stress treatment and the lowest seed yield (297.31 kg/ha) belonged to non-foliar application in severe water deficit stress. Water deficit stress increased the role of salicylic acid in fennel seed yield (Figure 2). Under stress conditions, methanol and salicylic acid application significantly increased seed yield compared to control treatment (non-foliar application) and spermidine application. There was no significant difference between control (non-foliar application) and spermidine foliar application treatments in all water deficit stress treatments. In general, methanol foliar application under non-stress conditions and salicylic acid under stress conditions had the highest seed yield (Figure 2). Bakhtati *et al.* (2016) showed that seed yield of *Cuminum cyminum* increased with the application of spermidine under drought stress but the increase in seed yield was not significant compared to the control (no use of spermidine). Salicylic acid significantly increases plant yield and yield components by regulating physiological and biochemical processes during plant growth in the face of biotic and abiotic stresses (Seagate *et al.* 2020). Salicylic acid accelerates growth improvement after water deficit stress by developing plant resistance to water deficit stress such as increased proline accumulation (Salarpour & Frahbakhash 2014). Ghilavizadeh *et al.* (2019) introduced a positive and significant role of salicylic acid foliar application in water deficit stress conditions on fennel seed yield. In many plants, increased seed yield has been reported due to methanol consumption (Faver & Gerik 2006; Moghadas *et al.* 2013).

Harvest index

Fennel harvest index was affected by the simple effects of water deficit stress and natural regulators,

but the interaction of these treatments on this trait was not significant (Table 3). The highest harvest index (19.51%) was observed in the moderate water deficit stress (irrigation treatment of 50% of field capacity), which did not have a significant difference with the non-water deficit stress condition (18.90%). The lowest harvest index (13.24%) was obtained in severe water deficit stress treatment (irrigation at 20% of field capacity), which had a significant difference between non-water deficit stress and moderate water deficit stress treatments (Table 4).

Water plays an important role in the transfer of materials to the seeds (Roghayyeh *et al.* 2014). The stress of water shortage during seed filling reduces the transfer of photosynthetic material to the seeds, which leads to a decrease in harvest index (Liu *et al.* 2017; Ghilavizadeh *et al.* 2019). Comparison of the mean effect of natural regulators on harvest index showed that the use of salicylic acid, and methanol caused a significant increase in fennel harvest index compared to non-foliar treatment and the application of spermidine was not significantly different from the control. Foliar application of methanol, salicylic acid and spermidine increased the harvest index compared to the control (non-foliar application) by 11.60%, 16.19%, and 1.42%, respectively (Table 4).

Essential oil percent

The percentage of seed essential oil under the water deficit stress was significant at the level of one percent probability (Table 3). The percentage of plant essential oil increased significantly with increasing water deficit stress (Table 4). So that, the lowest (2.56%) and the highest (3.98%) essential oil percentages were obtained in the treatment of non-stress and severe water deficit stress, respectively. The results of this study show that moderate and severe water deficit stresses increase significant essential oil percent in comparison with the non-stress condition.

With the availability of water and nutrients for the plant, the plant preferentially allocates carbon for growth, but with the reduction of irrigation water, the availability of nutrients is reduced and plant growth is limited more than photosynthesis, as a result, most of the photosynthetic material is allocated to the production of secondary metabolites and es-

sential oils (Bettaieb *et al.* 2009; Corell *et al.* 2012). Essential oils are part of the secondary metabolites in plants and plants increase the amount of these substances in their organs when receiving environmental stress (Diao *et al.* 2014; Ibrahim *et al.* 2020). Therefore, in the present experiment, water deficit stress condition increased the secondary metabolites of fennel, including the percentage of essential oil.

Essential oil yield

Essential oil yield was affected by the interaction effects of water deficit stress and natural regulators at a probability level of 5% (Table 3). In all foliar application treatments, the essential oil yield decreased with water deficit stress (Figure 3). Under non-water deficit stress conditions, methanol foliar application had the highest essential oil yield (22.67

Figure 3. Interaction of water deficit stress and natural regulators on essential oil yield of *Foeniculum vulgare*
Note: Means with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

Figure 4. Interaction of water deficit stress and natural regulators on proline content of *Foeniculum vulgare*
Note: Means with the same letter are no significant difference $P \leq 0.05$ based on Duncan's multiple range test

kg/ha). However, in moderate and severe water deficit stresses, the application of salicylic acid had the highest yield of essential oil with 21.25 and 11.86 kg/ha, respectively. The essential oil yield of fennel in moderate water deficit stress with the application of salicylic acid was not significantly different in comparison to methanol application with non-stress conditions (Figure 3).

Foliar application of methanol and salicylic acid under water deficit stress had a positive effect on essential oil yield, but the foliar application of salicylic acid performed better than methanol treatment and salicylic acid increased the yield of essential oil by more than 2 times in severe water deficit stresses (irrigation treatment of 50% of field capacity) in comparison to non-foliar application. In general, methanol foliar application under non-stress conditions and salicylic acid foliar application under water deficit stress conditions are recommended to achieve the highest essential oil yield. Essential oil yield is a result of the percentage of essential oil and seed yield (Diao *et al.* 2014). So the main reason for the high yield of essential oil in non-water deficit stress conditions and the use of methanol or salicylic acid is the higher seed yield in these treatments.

Although the percentage of essential oil increased in water deficit stress treatments, however, the yield of essential oil decreased due to a significant decrease in seed yield (Bahreininejad *et al.* 2014). Askari and Ehsanzadeh (2015) in *Foeniculum vulgare*, Castro *et al.* (2020) in *Lippia alba*, and Bettaieb *et al.* (2009) in *Salvia officinalis* reported drought stress significantly reduced essential oil yield.

Proline content

The interaction of water deficit stress and natural regulators significantly affected ($P \leq 0.05$) the proline content of the fennel (Table 3).

The amount of proline increased significantly in all foliar spraying treatments under water deficit stress conditions (Figure 4). The results confirmed a direct relationship between water shortage stress and increased proline content. Increasing the amount of proline in the cytoplasm of the plant causes osmotic regulation, maintenance of cell turgor pressure, and reduction of cell membrane damage, and also proline is a source of energy, carbon,

and nitrogen in plants (Tardieu *et al.* 2018). Proline prevents damage to the cell structure under water deficit stress (Solanki & Sarang 2014) and enzymes are protected by this proline mechanism due to their protein structure (Singh *et al.* 2017). Under non-stress conditions, proline content varied between 25.91 to 29.34 $\mu\text{mol/g}$ of fresh weight and the difference between natural regulators treatments was not significant (Figure 4).

In moderate water deficit stress (irrigation at 50% of field capacity), proline content in treatments of methanol (47.85 $\mu\text{mol/g}$ fresh weight) and salicylic acid (49.27 $\mu\text{mol/g}$ fresh weight) was significantly higher than the control (36.49 $\mu\text{mol/g}$ fresh weight) and the spermidine (38.16 $\mu\text{mol/g}$ fresh weight) treatments (Figure 4). In severe water deficit stress (irrigation at 20% of field capacity), proline content in the control treatment (57.71 $\mu\text{mol/g}$ fresh weight) was significantly lower than that in salicylic acid (72.38 $\mu\text{mol/g}$ fresh weight) and methanol (70.09 $\mu\text{mol/g}$) treatments. It has been reported that a positive relationship between plant proline content in water-deficit stress conditions with salicylic acid (Solanki & Sarang 2014) and methanol (Hossinzadeh *et al.* 2015). Methanol increased the activity of proline-5-carboxylate synthase (P5CS) by decreasing the pH in the plant and finally caused the accumulation of proline in the plant (Dorokhov *et al.* 2015; Hossinzadeh *et al.* 2015). The role of salicylic acid is approved as a growth regulator in tolerance to water deficit stress (Rizwan *et al.* 2020).

CONCLUSIONS

The results showed that the irrigation rate from 90% of field capacity (non-water deficit stress) to 20% of field capacity (severe water deficit stress) significantly reduced plant height, yield components, biological yield, seed yield, and essential oil yield. But the percentage of essential oil and the amount of proline increased significantly with water deficit stress. Examination of natural regulators treatments showed that the highest harvest index (18.73%), essential oil percent (3.31%), and plant height (74.35 cm) were obtained by foliar application with salicylic acid. Interaction effect of water deficit stress and natural regulators showed that methanol foliar

application under non-stress condition (irrigation at 90% of field capacity) had the highest number of branches per plant (15.28), number of umbels per plant (29.67), number of seeds per umbel (245.60), weight of one thousand seeds (5.93 g), biological yield (5,426.92 kg/ha), seed yield (1,127.59 kg/ha) and essential oil yield (22.67 kg/ha). The role of salicylic acid in reducing the negative effect of water deficit stress and improving the measured traits in fennel was significantly greater than the effect of methanol, so in water deficit condition, the highest amount of traits was obtained in foliar application with salicylic acid. According to the results of this study, the foliar application of salicylic acid in the cultivation of fennel under water stress is recommended for farmers.

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Received: November 11, 2020

Accepted: April 21, 2021