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FOLIAR SPRAY OF SALICYLIC ACID IMPROVED YIELD PARAMETERS AND ESSENTIAL OIL PRODUCTION OF DILL UNDER WATER DEFICIT

SUMMARY

Drought stress causes physiological disorders and growth restriction, leading to a decrease in crop productivity in the field. Foliar spray of salicylic acid may reduce the adverse effects of this stress on plant performance. Thus, this experiment was laid out as split plot with RCB design in three replicates to assess changes in morpho-physiological traits and essential oil production of dill (*Anethum graveolens* L.) seeds in response to irrigation intervals (water supply after 70, 100, 130, 160 mm evaporation) and foliar spray of water (control) and salicylic acid (0.6, 1.2 mM). The results showed that, plant height, plant biomass, seeds per umbel and plant were diminished due to water deficit, leading to a decline in seed yield. 1000-seed weight was slightly increased under stress, indicating the extent of drought tolerance of dill plants. Application of salicylic acid improved plant height, branches per plant, leaves per plant, umbels per plant, 1000-seed weight, plant biomass and finally seed yield under different irrigation intervals, especially under water limitation. Essential oil content and yield of seeds were increased with decreasing water supply up to moderate stress. The percentage and yield of essential oil were enhanced up to 54 days after flowering, and thereafter were slightly reduced. In general, application of 1.2 mM salicylic acid on non-stressed and drought-stressed plants was the superior treatment to improve field performance and essential oil production of dill. Therefore, drought tolerance and performance of plants can be promoted by salicylic acid treatment.

Keywords: dill; essential oil; harvest time; salicylic acid; seed yield; water stress

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INTRODUCTION

Medicinal plants are considered as valuable medicines in the world (Salmeron-Manzano *et al.,* 2020). Plants produce numerous secondary metabolites, known as allelochemicals that are involved in biochemical defense mechanisms. Most of them can be used as herbicide and pesticide (Yamada *et al.*, 2019). Plant Secondary metabolites are increasingly important factors in plant diversity and evolution (Mohammadi *et al.*, 2020). Essential oils are important secondary metabolites that are used worldwide to produce perfumes, cosmetics, drinks, medicines, fungicides, and insecticides (Chouhan *et al*., 2017).

Anethum graveolens known as dill is an annual herb of Umbels (*Apiaceae*) family, native to the Mediterranean and western Asia (Kharadi *et al*., 2019). The dill fruit has paired carpels (schizocarps), which are released at maturity (Ghassemi-Golezani *et al.*, 2016). Most of the dill essential oil is produced in seeds and flowers, although leaves and stems contain essential oil (Dimov *et al.*, 2019). The fruits of dill contain essential oil comprising of major compounds such as carvone (58 %), limonene (37%), α-phellandrene (36%), and limonene (31 %) in the leaves and in the seeds (Saıd-Al Ahl and Omer, 2016).

Studies have shown that essential oil of dill seeds has antiseptic, anti-carcinogenic, anti-hyperlipidemic properties, relieves intestinal spasms and griping, and helps to settle colic (Chahal *et al.*, 2017). Seed development is a series of events involving cell division, cell differentiation, and macromolecular storage. In cotyledons, cell differentiation begins in some parts and gradually spreads to other parts, thus building up a gradient for development. Seeds accumulate starch, store proteins, and oil in various proportions, depending on species and environmental conditions (Sagun *et al.,* 2023). Adverse environmental conditions may cause various responses in medicinal plants such as changes in growth and secondary metabolites (Isah, 2019). Major environmental stresses affecting crop performance are biotic or abiotic. Water shortage is the most important stress factor that limits crop growth and production via disruption of physiological and metabolic activities (Ahmad *et al.*, 2022). Reduction in cell turgor is the first sign of drought stress in many plants, which decreases plant growth and development. Plants tend to reduce transpiration under water shortage by decreasing the number and size of the leaves (Zhou *et al.*, 2021). Increasing drought stress significantly reduced the number of branches and seed yield in coriander plants. Water limitation during flowering and seed filling stages increased flower abortion, thus decreasing the seeds number per plant (Yeganehpoor *et al*., 2019). Reduction in seeds number per plant was also due to a decrement in flower formation (Benezit *et al.*, 2017). Drought stress during reproductive stages can decrease flowering and seed filling periods, that lowers the number and weight of seeds per plant (Jamshidi Zinab *et al.*, 2022). It was reported that irrigations after 130 and 160 mm evaporation caused a significant decrease in the yield components and seed yield of the safflower, compared to the control plants. While there was no significant difference between irrigations after 70 and 100 mm evaporation (Ghassemi Golezani *et al*., 2022). The essential oil percentage of plant organs increases with increasing water deficit during plant growth and development, but the essential oil yield decreases as a result of a large reduction in organs yield (Ghassemi-Golezani and Solhi-

Khajemarjan, 2021). Another well-adaptive response of plant species to drought stress is the rise in osmolytes such as soluble sugars and proline (Sharma *et al.*, 2019). The essential oil content of dill seeds may increase with decreasing water supply, leading to a highest essential oil yield in mild and moderate drought stresses (Ghassemi-Golezani *et al*., 2016).

Exogenous application of signaling molecules such as salicylic acid (SA) can reduce some of the detrimental impacts of drought stress on plants. Salicylic acid is an endogenous phenolic compound that regulates plant physiological and biochemical processes to improve growth, photosynthesis and productivity. This phytohormone modulates the syntheses of osmolytes and secondary metabolites to protect plants from adverse conditions. The SA induces enzymatic and non-enzymatic antioxidant activities in plants (Farhadi and Ghassemi-Golezani, 2020). A moderate concentration of SA may improve the antioxidant capacity of the plants under stress; but a higher rate of this hormone may limit plant growth (Liu *et al.,* 2022). Foliar application of SA enhances branches per plant and seed yield of coriander under different levels of water supply (Yeganehpoor *et al.*, 2019). This natural regulator causes a considerable increment in essential oils extracted from young shoots and peels of grapefruit (Khalid *et al*., 2018). Seed production of okra Plants treated with 1.2 mM salicylic acid was enhanced by 11.13%, compared to control plants (Rodrigues da Silva *et al*., 2023). Application of about 1.3 mM salicylic acid on tomato plants under drought stress reduced flower shedding and increased plant production by increasing gas exchange (Aires *et al*., 2022). Foliar application of 1 mM SA also increased photosynthetic pigments, plant biomass and essential oil production in Thymus vulgaris (Miri *et al*., 2015). Thus, this research was designed to examine changes in morphology, yield parameters, and essential oil production of dill plants in various levels of water stress and salicylic acid treatments.

MATERIAL AND METHODS

Location and experimental design

This research was conducted in 2019 at the Research Farm of the University of Tabriz, Iran, to evaluate essential oil accumulation in seeds of SA untreated (SA0: water spray) and treated (SA1: 0.6 and SA2: 1.2 mM) plants under different watering levels (I1, I2, I3, I4: irrigation after 70, 100, 130, 160 mm evaporation from a class A pan as normal irrigation and mild, moderate and severe stresses, respectively). The experimental work was performed as split-plot with randomized complete block design in three replications. Irrigation intervals and salicylic acid levels were allocated to main and sub plots, respectively. Each plot had six rows with a length of 5 m and a distance of 25 cm. Dill seeds (Tabriz ecotype) were treated with 2 g kg^{-1} Benomyl and then were seeded (80 seeds m^{-2}) at a depth of 1.5 cm of sandy loam soil. The plots were regularly irrigated from sowing up to seedling establishment, and subsequent irrigations were performed in accordance with the treatments. The weeds within the plots were removed at different stages of plant growth and development. The plants were sprayed by water (control) and SA at vegetative (48 days after sowing) and flowering (80 days after sowing) stages.

Measurements

At full flowering stage, 10 plants from each plot were harvested and plant height, leaves per plant, and branches per plant were determined.

At maturity, plants in 1 m^2 of each plot were harvested and number of umbels per plant, 1000-seed weight and seed yield were recorded. Then, seeds per umbel and seeds per plant were calculated. These plants were then dried in an oven at 80° C for 48 hours and above ground biomass was determined.

At each of six stages of seed development (26, 33, 40, 47, 54 and 61 days after flowering) 30 plants from each plot were harvested and the seeds were detached from plants. The seed samples were then dried at a room temperature of 20-25 ºC for 14 days. A sub-sample of 30 g powdered seeds from each plot was well mixed with 500 ml double-distilled water, and then the essential oil was extracted by hydro-distillation at 250 ºC for 3 h, using a Clevenger (Wang and Zhang, 2020). The essential oil percentage and yield were calculated as:

Essential oil percentage = (essential oil weight/seed weight) ×100

Essential oil yield (g m^{-2}) = essential oil percentage × seed yield (g m^{-2})

Statistical analysis

Analysis of variance of data was performed appropriate to experimental design, but essential oil accumulation at different stages of seed development was analyzed as split-split plot with randomized complete block design, using MSTAT-C software. The data means were compared following the Duncan multiple range test at $p \leq 0.05$. The figures were drawn by Excel software.

RESULTS AND DISCUSSION

Morphological traits and plant biomass

Data analysis showed (Table 1) that plant height and plant biomass were significantly affected by irrigation intervals and SA levels. Leaves and branches per plant were only affected by SA, and leaf water content was only influenced by water stress.

Table 1. Analysis of variance of the data for morphological traits of dill affected by water supply and salicylic acid

Treatments	df	Mean squares			
		Plant height	Branches per plant	Leaves per plant	Plant biomass
Replication	2	4.43	0.21	0.42	2102.90
Irrigation (I)	3	511.14**	6.90	2.07	51760.47**
Ea	6	5.33	5.57	1.25	2048.34
Salicylic acid (SA)	2	52.22**	7.23**	7.10**	36737.08**
I × SA	6	11.38	0.52	0.34	3592.46
Eb	16	4.25	0.83	0.23	1484.90
CV (%)	-	4.22	22.50	9.19	8.60

***, significant p ≤ 0.01, respectively*

The highest plant height and plant biomass were obtained under normal irrigation (I1) and decreased with increasing water stress (Table 2). The greatest improvement in plant biomass (about 23%), plant height (about 9%), branches per plant (about 52%) and leaves per plant (29%) was achieved by 1.2 mM SA, followed by 0.6 mM SA, although there was no significant difference between 0.6 mM treated and untreated plants.

Table 2. Average plant height, branches per plant and leaves per plant of dill under different irrigation treatments in response to salicylic acid

Treatments	Plant height (cm)	Branches per plant	Leaves per plant	Plant biomass (g m^{-2})
Irrigation (I)				
I_1	59.71±0.50 a	--	--	517.85±7.59 a
I_2	48.12±1.37 b	--	--	482.30±20.03 a
I_3	43.97±1.64 c	--	--	449.00±30.68 a
I_4	43.54±0.60 c	--	--	342.18±14.55 b
Salicylic acid (SA)				
SA_0	47.07±2.16 b	2.16±0.33 b	4.56±0.15 b	403.83±24.39 b
SA_1	48.30±2.04 b	2.59±0.47 ab	5.10±0.25 b	429.72±27.70 b
SA_2	51.14±2.16 a	3.67±0.41 a	6.10±0.34 a	509.95±26.09 a

Different letters in each column indicate significant difference at $p \leq 0.05$ (Duncan test)

Yield components and seed yield

Irrigation intervals and salicylic acid levels showed significant effects on umbels per plant, seeds per umbel, 1000 seed weight and seed yield of dill (Table 3). The seeds per plant was only affected by irrigation treatment. The interaction of water stress × salicylic acid was not significant for all these traits.

Table 3. Analysis of variance of the effects of water stress and salicylic acid on yield components and seed yield

Treatments	df	Mean squares				
		umbels per plant	Seeds per umbel	Seeds per plant	1000 Seed weight	Seed yield
Replication	2	0.61	1461.88	109902.43	1.83	92.57
Irrigation (I)	3	3.52*	168947.05**	2641140.84**	21.17**	18691.22**
Ea	6	0.45	2084.26	35360.50	0.41	335.27
Salicylic acid (SA)	2	4.06**	28993.26**	55199.89	2.88**	8759.20**
I × SA	6	0.50	1416.12	31717.12	0.15	582.54
Eb	16	0.30	2740.23	26234.52	0.09	218.36
CV (%)	-	13.25	20.34	16.01	9.61	7.01

**, **: significant at $p \leq 0.05$ and $p \leq 0.01$, respectively*

The mean number of umbels per plant (about 21%) was significantly decreased under severe water deficit, but no significant decline of this traits was observed under mild and moderate drought stresses (Table 4). The seeds per umbel (about 39%) and per plant (about 61%) were significantly reduced under all limited irrigation, compared to normal irrigation. No significant differences were observed in umbels per plant between mild and moderate stresses and in seeds per plant among all stress treatments. The largest seeds were produced under mild stress, which had no significant difference with moderate stress. The 1000 seeds weight was statistically similar for normal irrigation and severe stress. Seed yield per unit area (up to 51%) was significantly decreased under moderate and severe stresses. However, there was no significant difference between normal irrigation and mild stress. Foliar spray of salicylic acid, particularly with 1.2 mM concentration, significantly enhanced, umbels per plant (about 28%), seeds per umbel (about 35%), 1000 seed weight (about 31%) and seed yield per unit area (about 24%).

Table 4. Means of plant biomass, yield components and seed yield of dill affected by irrigation intervals and salicylic acid levels

Treatments	Umbels per plant	Seeds per umbel	Seeds per plant	1000 seed weight (g m^{-2})	Seed yield (g m^{-2})
Irrigation (I)					
I_1	4.14±0.28 ab	435.87±35.60 a	1794.20±131.01 a	1.76±0.05 b	248.21±4.70 a
I_2	4.14±0.99 ab	160.19±29.40 c	633.32± 127.24 b	4.86±0.33 a	237.56±10.12 ab
I_3	4.90±0.95 a	140.20±24.23 c	662.54±97.38 b	4.04±0.24 a	211.21±14.65 b
I_4	3.36±0.69 b	293.17±35.89 b	957.04± 158.95 b	1.97±0.14 b	146.64±12.64 c
Salicylic acid (SA)					
SA_0	3.52±0.23 b	219.54±49.09 b	--	2.68±0.35 c	189.50±14.44 b
SA_1	4.20±0.32 a	239.61±60.49 b	--	3.13±0.46 b	201.94±11.09 b
SA_2	4.68±0.27 a	312.92±63.43 a	--	3.66±0.47 a	241.26±7.63 a

Essential oil accumulation and yield

Essential oil percentage and yield of dill seeds were significantly affected by irrigation intervals, salicylic acid levels and harvest time (Table 5). The interaction of irrigation × harvest time was also significant for both traits.

Table 5. Analysis of variance of the data for essential oil percentage and yield of dill seeds affected by irrigation intervals and salicylic acid levels

Treatments	df	Mean squares	
		Essential oil percentage	Essential oil yield
Replication	2	0.40	2107.01
Irrigation (I)	3	1.62*	5489.21**
Error (I)	6	0.16	392.25
Salicylic acid (SA)	2	3.17**	6678.78**
I × SA	6	0.05	328.47
Error (SA)	16	0.02	132.96
Harvest time (T)	5	3.11**	33413.63**
I × T	15	0.17**	944.52**
SA × T	10	0.03	434.02
I × SA × T	30	0.01	325.52
Error (T)	120	0.06	236.41
CV (%)	-	19.80	21.98

**, ** significant at $p \leq 0.05$ and $p \leq 0.01$, respectively*

The highest essential oil percentage and yield were recorded under mild stress, followed by moderate stress (Table 6). There was no significant difference in essential oil percentage among different levels of stress. However, essential oil yield under severe stress was lower than other stress levels. Salicylic acid enhanced essential oil percentage (up to 32%) and yield (up to 25%), but to improve the essential oil percentage of dill seeds, 1.2 mM SA was the superior treatment.

Table 6. Means of essential oil percentage and yield for irrigation intervals and salicylic acid levels

Treatments	Essential oil percentage	Essential oil yield
Irrigation (I)		
I_1	1.01±0.02 b	19.59±1.45 b
I_2	1.37±0.02 a	25.80±2.01 a
I_3	1.38±0.05 a	23.76±1.42 ab
I_4	1.33±0.03 a	19.31±1.75 b
Salicylic acid (SA)		
SA_0	1.09±0.03 c	19.95±2.87 b
SA_1	1.23±0.09 b	20.80±2.67 b
SA_2	1.50±0.17 a	25.60±2.86 a

Different letters in each column indicate significant difference at p ≤ 0.05 (Duncan test)

With increasing seed development, the essential oil percentage of dill seeds was increased up to 54 days after flowering, and thereafter no tangible changes were observed. The essential oil content of seeds at all harvest times under normal irrigation was lower than stress levels. The highest essential oil percentage at final harvest was recorded under moderate stress (Figure 1A).

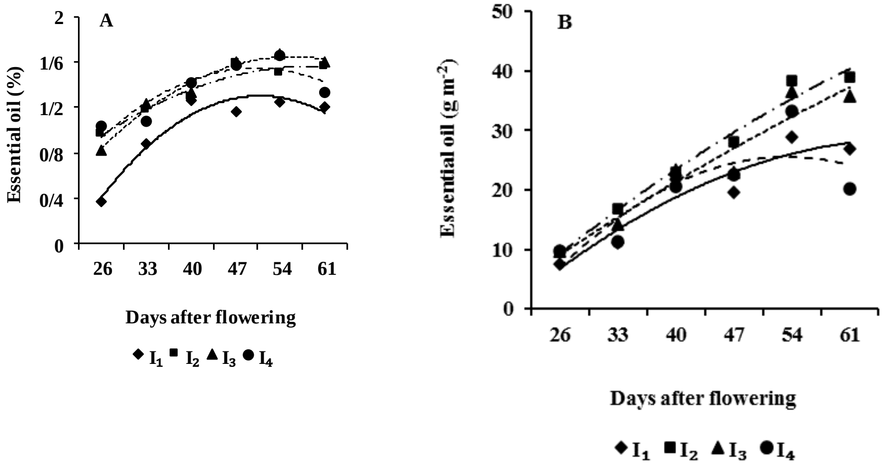

Figure 1. Changes in essential oil percentage (A) and yield (B) of dill seeds during development in response to irrigation

In contrast, essential oil yield per unit area under normal irrigation and mild and moderate stress levels was enhanced up to final harvest (61 days after flowering). However, under severe stress it was increased up to 54 days after flowering and after that it was slightly decreased. At the early stages of seed development, there was a little difference in essential oil production among irrigation intervals, but these differences were enhanced at subsequent harvests, particularly at seed maturity stage. The highest essential oil yield of dill seeds at this stage was obtained under mild stress, followed by moderate stress (Figure 1B).

CONCLUSIONS

Increasing irrigation intervals was led to a decrease in plant height, plant biomass and consequently seed yield in dill plants. However, the highest essential oil percentage and yield was recorded under mild and moderate water deficits. Foliar spray of SA, particularly with 1.2 mM concentration decreased the adverse effects of water limitation and enhanced essential oil percentage and yield via improving plant growth, seed weight and yield. Therefore, this could be introduced as an effective treatment for improving field performance and essential oil production of dill plants under various levels of water stress. Future investigation on the impacts of drought and SA levels on different medicinal plants may support the findings of this research.

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