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Original Article

Influence of Salicylic Acid on Growth, Yield and Macro-elements Absorption of Fennel (*Foeniculum vulgare* Mill.) under Water Stress

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Abstract

The present study was conducted in order to investigate the effect of salicylic acid on growth, yield and macro-elements absorption of fennel in split-plot as base on randomized complete block design (RCBD) with three replications in Tehran, Iran. Irrigation regime as main plot was undergone in 5 levels that included: I_1: full irrigation, I_2: cut off irrigation at stem elongation stage, I_3: cut off at budding stage, I_4: cut off irrigation at 50% flowering stage and I_5: cut off irrigation at grain filling stage. Foliar application of SA (0, 3, 6, and 8 mM) as sub plot was applied at stem elongation, budding and 50% flowering stages. Result indicated application of SA was resulted to increase biological yield in all irrigation regimes. However, application of 6 mM SA had the most biomass under water stress at grain filling stage. Interaction of water stress and SA demonstrated that application of 3 mM SA had the most grain yield under full irrigation, water stress at stemming and water stress at grain filling stages, while application of 9 mM SA had the highest grain yield under water stress at budding and 50% flowering stages.

Keywords: Grain yield, Macro-elements, Medicinal herb, Salicylic acid, Water stress.

Introduction

Fennel is one of the oldest known spice plants, and people of ancient Roman and Greek have used it for disease treatment [1,2]. It is a perennial and aromatic herb which belongs to *Apiaceae* family [3, 4] with elliptical fruits, slightly curved and pale grayish green in color [5]. It is generally considered indigenous to Europe and the shores of the Mediterranean [6]. All organs of fennel contain volatile oil and its seed is the most important organ in oil producing. Oil percent is variable and depends on ecological factors [7]. Among its compounds, anetholeis often the most important one [8, 9] which has proper antioxidant activity [10]. Extract of different parts of fennel is able to neutralize free radicals produced by oxidation [11, 12]. Its active substances are used in pharmacy for treatment of cough, abdominal pain, flatulence, dyspepsia and induction of lactation [8].

Plant and climate diversity in Iran caused notation to the cultivation of suitable medicinal herbs in each region additionally by planning for traditional utilization of forest and rangeland areas [13]. On the other hand, drought is the most common environmental stress in Iran [14] and its effect on plants growth and yield depends on their genotypes [15], stress duration, durability and rate of water shortage [16]. It should be noted that fennel wildly grows in arid and semiarid regions (soils with high concentration of solutes) [1,2]. Water deficit on fennel led to reduction in plant height, main stem number and fruit yield [17]. Further irrigation caused significant improve in fennel height, lateral-

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stem number and fruit yield [18]. Various drought and nitrogen levels on the plant growth, oil and fruit yield indicated that stress significantly influenced these factors and drought stress in flowering stage caused reduction in plant height, stem diameter, main-stem number, fruit yield, oil yield and water use efficiency [19].

Various concentrations of SA effects on *Anethum graveolens* L. have been evaluated by Abdi *et al*. [21] and plant height, density, fresh and dry weight of root, stem diameter, leaf number, and fresh and dry weight of shoot in SA treatments were higher than control treatment. Moreover, low concentrations of this growth regulator were necessary for obtaining desired results. Application of water deficit treatments and possibility of producing plants with higher water use efficiency can lead to medicinal plants producing with high quality and quantity in the long term additionally by increasing area under cultivation. Therefore, the aim of present study was investigated the effect of salicylic acid foliar application on growth and some agronomic traits of fennel under irrigation regimes in Robat-Karim region.

Material and Methods

This study was performed during 2015 in split-plot experiment based of randomized complete block design (RCBD) with three replications in Robat Karim city (longitude, 51°4'52.82"E; latitude, 35°28'6.37"N and altitude1050 m) which is located at southwest of Tehran Province, Iran. Irrigation regime as main plot was undergone in 5 levels that included: I_1: full irrigation, I_2: cut off irrigation at stem elongation stage, I_3: cut off at budding stage, I_4: cut off irrigation at 50% flowering stage and I_5: cut off irrigation at grain filling stage.

Foliar application of SA (0, 3, 6, and 8 mM) as sub plot was applied at stem elongation, budding and 50% flowering stages.

Experimental plots ($6m^2$) were prepared as ridge and furrow form, which intervals between blocks, main plots, subplots, ridges of each plot and plants on ridge were 3 m,2 m, 2 m, 50 cm and 40 cm, respectively. The seeds were wild (Landrace) that collected from Hashtgerd area (longitude, 50° 43' E; latitude, 35° 65' N and altitude 1426 m) in Alborz province. Seeds were sown in 25 March. Plants were thinned at 10-leaves stage. Irrigation was done by waterlogging method and cut off irrigation was applied as described above.

F. vulgare was harvested at fruit maturity stage (10 September) when the seeds were brown and splitting in two mericarps. Measured parameters included: plant height, stem diameter, canopy perimeter, lateral branch number, umbrella number per plant and per main branch, grain number per umbel, nitrogen, phosphorus, potassium concentration, biological yield, grain yield, essential oil content and essential oil yield.

To measurement of macro-elements, the Hydrochloric acid combination was applied that its extract in order to measurement of nitrogen by Kjeldahl method [22], phosphorous by ammonium vanadate colorimetric method [23], potassium by flame photometer device [24] were done. The extracted essential oil was analyzed by GC-MS (Thermo Quest2000) [25].

Data were analyzed by using Statistical Analysis System (SAS, ver 9.1.3). Significant differences in means between the treatments were compared by Duncan's Multiple Range Tests (DMRT) by MSTAT-C software.

Table 1 Minimum and maximum data related temperature during 2016 in Robat Karim regions

Month	Robat Karim	
	Temperature maximum (ºC)	Temperature minimum (ºC)
March	15.5	3.7
April	21.8	8.7
May	29.2	14.4
June	33.9	18.5
July	36.6	21.1
August	36.2	21.1
September	31.4	16.2

Result and Discussion

Variance analysis indicated (Table 1) that water stress significantly affected all morphological traits. SA application was different for stem diameter, umbrella number per main branch and umbel number per umbrella at 1% probability level. There was significant effect of irrigation regime and SA interaction on stem diameter, canopy perimeter, lateral branch number, umbrella number per main branch, and grain number per umbrella.

Table 2 Result of physico-chemical analysis of farm soil

Region	Texture class	Sand (%)	Silt (%)	Clay (%)	K mg/kg	P mg/kg	Na mg/kg	C (%)	N (%)	Lime (%)	EC Ds/m	pH 1:25
Robat Karim	L	45	30	25	197.6	10.2	38.7	0.57	0.04	3.1	0.22	8.5

Cut off irrigation in different growth stages revealed that full irrigation significantly increased plant height (79.4 cm) and CI at grain filling stage umbel number per umbrella (13.4) (Table 2). Application of 6 mM SA increased umbel number per umbrella to 12.2, which was significantly higher than 8 mM SA application (Table 3).

Cut off irrigation at 50% flowering stage and no application of SA had significantly higher stem diameter than the other treatments. The highest canopy perimeter (152.6 cm) was with the CI at 50% flowering stage with 6 mM SA foliar application. There was the highest number of lateral branches (22) and umbrella per main branch (12.3) in cut off irrigation at grain filling stage and no application of SA. The highest number of umbrella per plant (31) and grain per umbel (16) observed with CI at grain filling stage and 3 mM SA (Table 4).

Water stress effects depend on plants genotypes [15], stress duration, durability and rate of water shortage [16], and it led to reduction in all of the morphological traits [17] which were studied in this project. Whereas, fennel is one of drought tolerant plants [1,2], thus it can be concluded that water stress influence whole plants (tolerant and non-tolerant). The maximum height obtained from FI treatment could be related to enlarging and multiplication of cells.CI at grain filling stage leading to maximum number of umbels per umbrella could be due to increasing concentration of cell sap. Water stress application at pollination and grain filling stages improved grain number of fennel. In general, these findings about effect of drought stress at various growth stages of fennel matched with others results [18,19]. The highest umbrella number per branch observed with 8 mM SA (32.3) reflects effectiveness of SA in increasing grain number [20]. Whereas, there was significant difference among various levels of SA and control on plant height, canopy perimeter and lateral branch number. Askari and Ehsanzadeh [26] reported that salicylic acid led to increases in root growth and yield attributes, seed essential oil content and yield, and irrigation water use efficiency aspects under both moisture conditions. Hashmi *et al* [27] also cited that the foliar spray of SA at 10^{-4} M significantly enhanced the vegetative growth (shoot and root lengths, fresh and dry weights), physiological and biochemical characteristics (chl ‘a’, chl ‘b’, total chlorophyll and carotenoids contents, nitrate reductase activity, carbonic anhydrase activity, leaf-N, -P and -K contents), yield characteristics (number of umbels and fruits, 1,000-seed weight and seed yield) and essential oil yield of fennel.

Drought stress had significant effect on the absorbed elements of fennel. Whereas, individual application of SA had no influence on the elements absorption unless nitrogen. There was significant interaction among water and SA on N, P and K absorption (Table 5).Water stress in different stages of growth had considerable effects on their absorption, so that CI at budding stage until flowering and full irrigation caused the highest absorption of N (Fig. 1.a).Application of SA could decrease the elements absorption that was few statistically significant for N. The highest percent of N was observed in no application of SA and 3 mM SA (Fig. 1b).The interaction of treatments revealed that no application of SA with full irrigation had the highest N and P value and no application of SA with CI at grain filling stage had the most K value (Table 6). The result of analysis variance (Table 7) showed that water stress, salicylic acid and their interaction had significant effect on biological yield, grain yield, essential oil content and essential oil yield. Result indicated application of SA was resulted to increase biological yield in all irrigation regimes. However, application of 6 mM SA had the most biomass under water stress at grain filling stage (Fig. 2). Interaction of water stress and SA demonstrated that application of 3 mM SA had the most grain yield under full irrigation, water stress at stemming and water stress at grain filling stages, while application of 9 mM SA had the highest grain yield under water stress at budding and 50% flowering stages (Fig. 3). Investigation of essential oil content result showed that application of 8 mM SA had the most essential oil content at full irrigation, water stress at stemming and 50% flowering stages.

Table 1 Variance analysis of irrigation regime and salicylic acid effects on morphological traits of *Foeniculum vulgare* Mill.

SOV	df	Mean squares							
		Plant height	Stem diameter	Canopy perimeter	Lateral branches number	Umbrella number per plant	Umbrella number per main branch	Umbel number per umbrella	Grain number per umbel
Replication	2	414.3**	0.001	3658.8**	9.8**	17.4*	10.5**	22**	13.6**
Irrigation regime (A)	4	1263.3**	0.02**	759.7**	71.9**	199.5**	45.9**	14.7**	12.05**
Error A	8	62.7	0.002	83.3	1.0	1.6	0.26	0.5	0.6
Salicylic acid (B)	3	64.8	0.04**	67.4	0.23	13.3	12.6**	6.9**	4.4
A × B	12	24.2	0.02**	437.8**	4.5**	14.5	5.7**	2.5	4.2*
Error	30	35.2	0.001	97	0.9	3.4	1.3	1.4	2
CV (%)		8.8	9.55	7.8	5.3	7.7	11.9	10.1	10.6

df, degrees of freedom; ns, not significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 2 Effect of irrigation regime on morphological traits of *Foeniculum vulgare* Mill.

Irrigation regime	Plant height (cm)	Stem diameter (cm)	Canopy perimeter (cm)	Lateral branches number per plant	Umbrella number per plant	Umbrella number per main branch	Umbel number per umbrella	Grain number per umbel
Full irrigation	79.4 a	0.33 c	117c	14.7 c	21 c	6.6 c	10.5 c	14.2 a
CI at stem elongation stage	73.4 b	0.4 b	127.7 b	18.3 b	24.6 b	9 b	11.4 bc	13b c
CI at budding stage	68.6 b	0.35 c	123.6 bc	15.5 c	25 b	9.4 b	12.08 b	13.6ab
CI at 50% flowering stage	63 c	0.44 a	137.4 a	19.5 a	19 d	11.3 a	11 c	11.8 c
CI at grain filling stage	52.5 d	0.4 b	119.9 bc	20.2 a	29.6 a	11.4 a	13.4 a	13.5 b

CI, cut off irrigation; means in a column followed by the same letter are not significantly different (P≤0.05).

Table 3 Effect of salicylic acid on morphological traits of *Foeniculum vulgare* Mill.

Salicylic acid (mM)	Plant height (cm)	Stem diameter (cm)	Canopy perimeter (cm)	Lateral branches number per plant	Umbrella number per plant	Umbrella number per main branch	Umbel number per umbrella	Grain number per umbel
0	66.4 a	0.4 a	124.2 a	17.6 a	23.3 bc	8.3c	11.7 a	12.8 a
3	64.9 a	0.3 b	128.2 a	17.6 a	25 a	9.4b	12 a	13.8 a
6	69.2 a	0.4 a	123.5 a	17.8 a	24.2 ab	10.2ab	12.2 a	12.8 a
8	69.2 a	0.3 b	124.5 a	17.6 a	22.8 c	10.3a	10.7 b	13.6 a

Means in a column followed by the same letter are not significantly different (P≤0.05).

However, the most essential oil content in filling stage was observed in non-application of SA (Fig. 4). On the other hand, interaction of water stress and SA on essential oil yield showed that application of 3 mM SA had the most essential oil yield under full irrigation, water stress at stemming and grain filling stages, while application of 8 mM SA had the most value under water stress at budding and 50% flowering stages (Fig. 5). Due to the considerable decrease in essential oil yield in cut off irrigation at budding and 50% flowering stages, increasing of SA concentration (8 mM) can be effective in decline the adverse of water stress.

SA plays an important role in the regulation of some physiological processes in plants such as effects on growth and development, ion uptake and transport and membrane permeability [28]. Miura and Tada [29] reported that the effects of SA on the physiological processes of plants depend on its concentration, type of plant, the stage of plant growth and environmental conditions. Generally, low concentrations of SA may enhance the antioxidant capacity and tolerance to abiotic stresses but high concentrations of SA may cause cell death or susceptibility to abiotic stresses [30].

Table 4 Interaction effect of irrigation regime and salicylic acid on morphological traits of *Foeniculum vulgare* Mill.

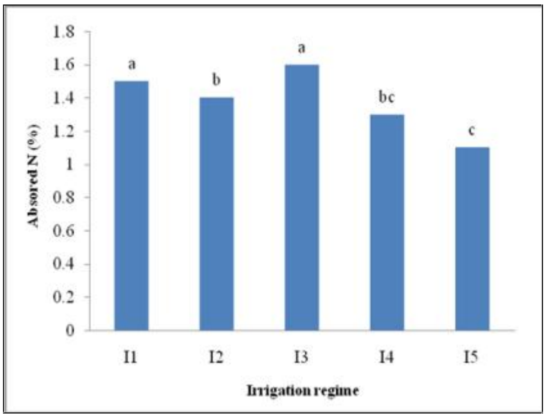
Irrigation regime	salicylic acid (mM)	Plant height (cm)	Stem diameter (cm)	Canopy perimeter (cm)	Lateral branches number per plant	Umbrella number perplant	Umbrella number per main branch	Umbel number per umbrella	Grain number per umbel
Full irrigation	0	78.3ab	0.3f	113.6efg	14 h	20.3 hij	5.3 h	11 b-e	14 a-d
	3	78.3ab	0.33ef	120c-g	14 h	21.6 ghi	7f gh	12 bcd	16 a
	6	78.6ab	o.4cde	120.6c-g	16.3 fg	22.6 e-h	8.6 def	10.3 cde	13 bcd
	8	82.3a	0.3f	113.6efg	14.6 gh	19.3 hij	5.6 h	9 e	15.3 ab
CI at stem elongation stage	0	74.3abc	0.46 bc	128.6 b-f	17 ef	24.6 c-g	6 gh	10.6 b-e	13 bcd
	3	72.6abc	0.36 def	128.6 b-f	18.3 de	27 bcd	8 efg	13 ab	15 abc
	6	77ab	0.5 ab	133 b-e	19.3 bcd	25.6 cde	10.3 a-d	12 bcd	12 de
	8	69.6bc	0.3 f	120.6 c-g	18.6 cde	21.3 g-i	11.6 ab	10 de	12 de
CI at budding stage	0	69bc	0.36 def	125.3 c-f	16 fg	24 d-g	8 efg	12 bcd	13 bcd
	3	64.6cde	0.3 f	126.6 c-f	15 gh	26.3 bcd	9 c-f	11.3 bcd	14 a-d
	6	70.6bc	0.3 f	109.6 fg	14 h	27.6 bc	10.6 a-d	13 ab	13 bcd
	8	70.3bc	0.46 bc	133 b-e	17 ef	22 f-i	10 b-e	12 bcd	14.6 a-d
CI at 50% flowering stage	0	63 cde	0.56 a	134.3 bcd	19 bcd	18j	10 b-e	12 bcd	12 de
	3	57 def	0.46 bc	145.6 ab	20 bcd	19ij	12 ab	11 b-e	10 e
	6	64.3 cde	0.43 bcd	152.6 a	20.3 bc	20hij	11 abc	11 b-e	13 bcd
	8	67.6 bcd	0.3 f	117 d-g	19 bcd	19ij	12.3 a	10 de	12.3 cde
CI at grain filling stage	0	47.6 f	0.5 ab	119.3 c-g	22 a	29.6 ab	12.3 a	13 ab	12 de
	3	52 f	0.33 ef	120.3 c-g	20.6 ab	31 a	11 abc	13 ab	14 a-d
	6	55.3 ef	0.5 ab	101.6 g	19.3 bcd	25.3 c-f	10.3 a-d	15 a	13 bcd
	8	55.3 ef	0.3 f	138.3 abc	19 bcd	32.3 a	12 ab	12.6 bc	14 a-d

Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 5 Variance analysis of irrigation regime and salicylic acid effects on absorbed elements of *Foeniculum vulgare* Mill.

SOV	df	Mean squares		
		N	P	K
Replication	2	0.13 **	0.34 **	0.17 **
Irrigation regime (A)	4	0.4 **	0.47 **	0.87 **
Error A	8	0.08	0.04	0.03
Salicylic acid (B)	3	0.08 *	0.07 ^{ns}	0.02 ^{ns}
A×B	12	0.02 ^{ns}	0.08 *	0.08 **
Error	30	0.02	0.03	0.01
CV (%)	-	11.1	9.5	10.1

ns, no significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.



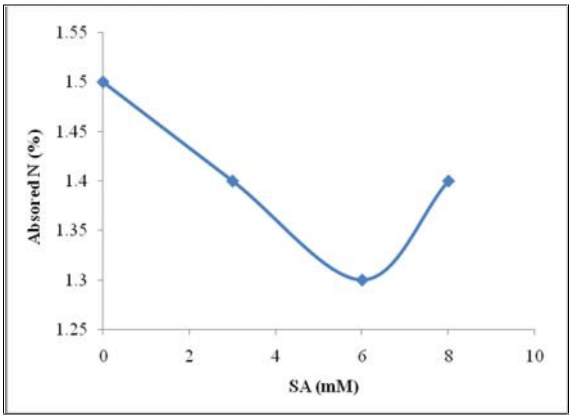


Fig. 1: a) Effect of irrigation regime b) Effect of salicylic acid on absorbed nitrogen. I_1: Full irrigated, I_2: Cut off irrigation at stemming, I_3: Cut off irrigation at budding stage, I_4: Cut off at 50% flowering stage and I_5: Cut off at early grain filling stage.

Table 6 Interaction effect irrigation regime and salicylic acid on absorbed elements of *Foeniculum vulgare* Mill.

Irrigation regime	salicylic acid (mM)	N (%)	P (%)	K (%)
Full irrigation	0	1.7 a	2.5 a	0.7 h
	3	1.5 a-d	1.7 ef	0.8 gh
	6	1.4 b-e	2.1 bc	0.7 h
	8	1.6 ab	2.2 b	0.9 fgh
CI at stem elongation stage	0	1.5 a-d	1.9 c-f	0.8 gh
	3	1.3 cde	2.03 b-e	0.9 fgh
	6	1.4 b-e	2.05 bcd	1 efg
	8	1.4 a-d	1.9 b-f	1.05 def
CI at budding stage	0	1.6 abc	1.8 c-f	1 efg
	3	1.7 ab	1.7 def	1.1 b-e
	6	1.4 a-d	1.7 def	1.2 bcd
	8	1.6 abc	1.7 def	1.2 bc
CI at 50% flowering stage	0	1.4 b-e	1.8 c-f	1.07 c-f
	3	1.3 cde	1.7 def	1.3 b
	6	1.2 de	1.6 f	1.2 bcd
	8	1.2 de	1.6 f	1.3 b
CI at grain filling stage	0	1.2 de	1.7 ef	1.7 a
	3	1.2 de	1.7 def	1.6 a
	6	1.1 e	1.7 ef	1.3 b
	8	1.2 de	1.6 f	1.1 b-e

Means in a column followed by the same letter are not significantly different ($P \leq 0.05$).

Table 7 Variance analysis of irrigation regime and salicylic acid effects on yield of *Foeniculum vulgare* Mill.

SOV	df	Mean squares			
		Biological yield	Grain yield	Essential oil content	Essential oil yield
Replication	2	709737.02 **	6164.25 **	0.27 **	2069.24 **
Irrigation regime (A)	4	7021415.21 **	64344.52 **	1.27 **	2349. **
Error A	8	3693.05	364.25	0.01	17.3
Salicylic acid (B)	3	296785.28 **	28714.69 **	0.07 **	234.90 **
A × B	12	40325.82 **	18884.32 **	0.05 **	117.6 **
Error	30	1988.5	282.30	0.006	14.6
CV (%)		13.01	12.42	12.30	13.4

df, degrees of freedom; ns, not significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$.

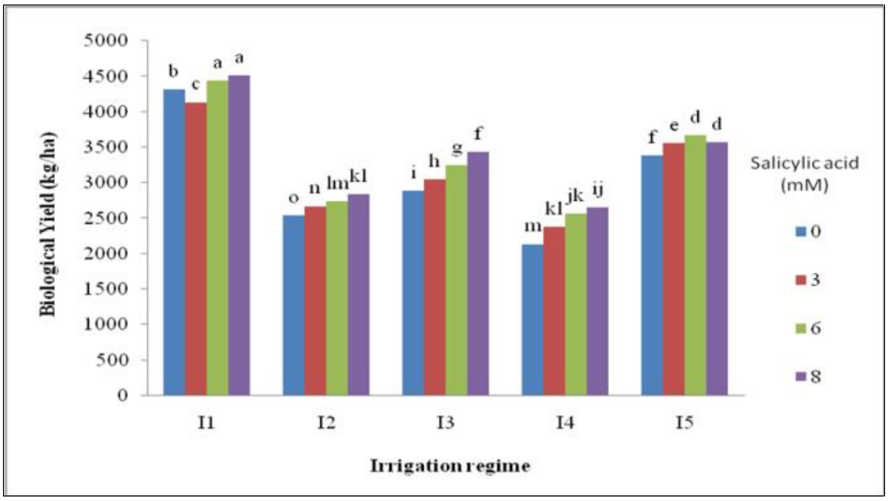


Fig. 2 Interaction of salicylic acid×irrigation regime on biological yield. I_1: Full irrigated, I_2: Cut off irrigation at stemming, I_3: Cut off irrigation at budding stage, I_4: Cut off at 50% flowering stage and I_5: Cut off at early grain filling stage.

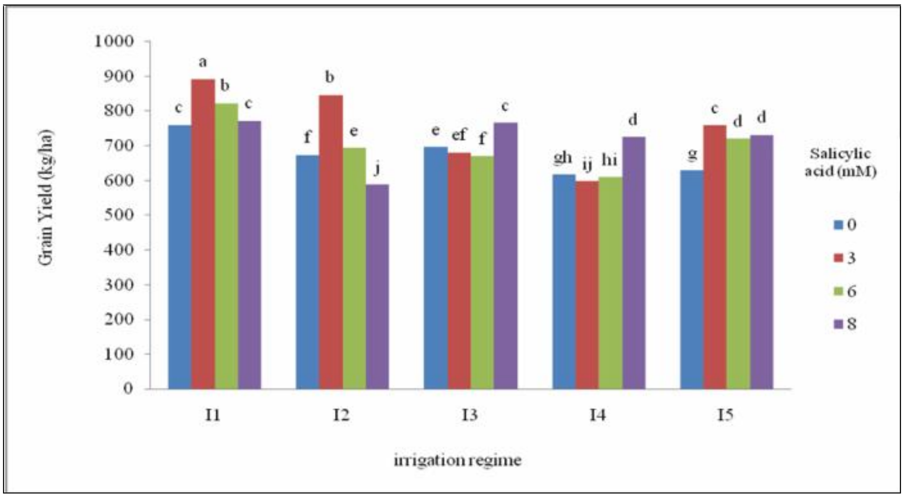


Fig. 3 Interaction of salicylic acid×irrigation regime on grain yield. I_1: Full irrigated, I_2: Cut off irrigation at stemming, I_3: Cut off irrigation at budding stage, I_4: Cut off at 50% flowering stage and I_5: Cut off at early grain filling stage.

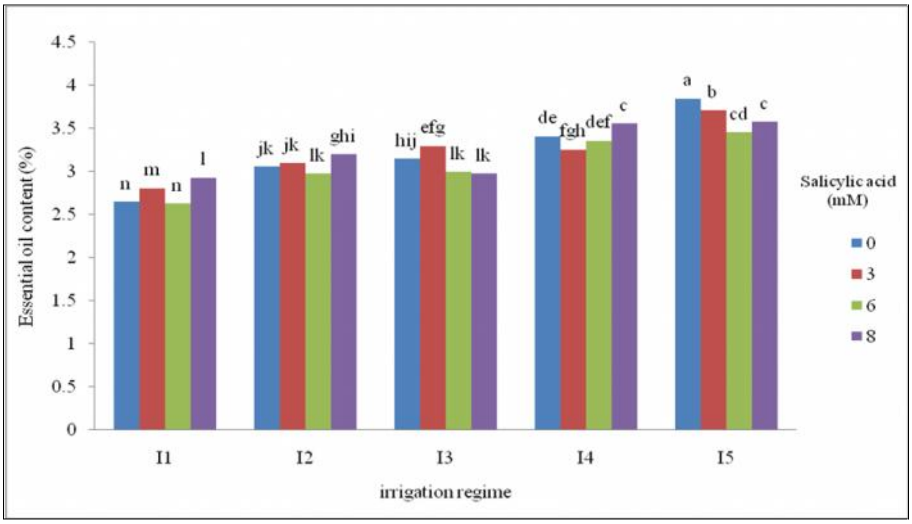


Fig. 4 Interaction of salicylic acid×irrigation regime on essential oil percent. I_1: Full irrigated, I_2: Cut off irrigation at stemming, I_3: Cut off irrigation at budding stage, I_4: Cut off at 50% flowering stage and I5: Cut off at early grain filling stage.

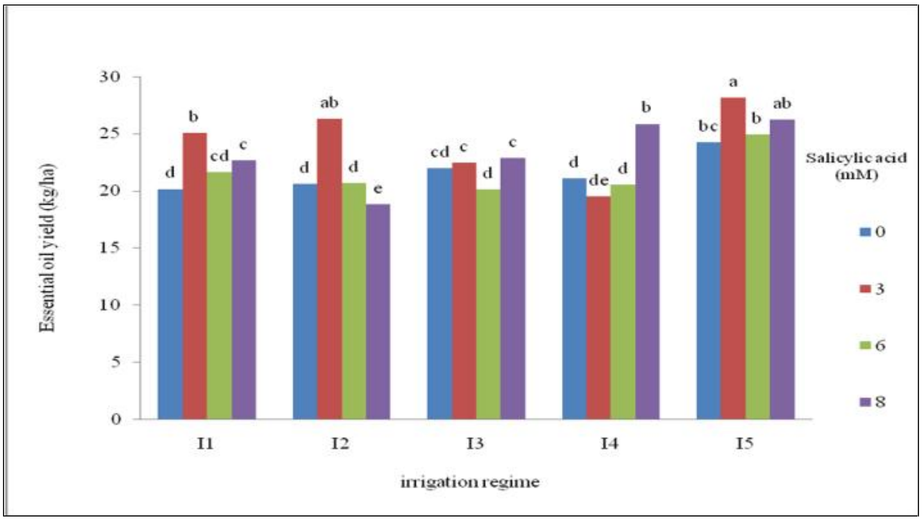


Fig. 5 Interaction of salicylic acid × irrigation regime on essential oil yield. I_1: Full irrigated, I_2: Cut off irrigation at stemming, I_3: Cut off irrigation at budding stage, I_4: Cut off at 50% flowering stage and I_5: Cut off at early grain filling stage.

Conclusion

In general, the highest grain yield and biological yield obtained with full irrigation. Application of 3mM SA under full irrigation condition had the most grain yield of fennel plants. Result also indicated application of 3 mM SA under cut off irrigation at early grain filling had the highest essential oil yield. Application of 8 mM SA in severe waters stress i.e. budding and 50% flowering stage can be effective for relative compensation of stress damage.

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