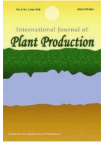

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Improving essential oil content and yield of ajowan organs under water stress by application of salicylic acid and abscisic acid

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Abstract

This research was carried out in 2014 and 2015 to assess the effects of salicylic acid (SA) (0 and 1 mM) and abscisic acid (ABA) (0 and 50 µM) on essential oil content and yield of ajowan (*Carum copticum* L.) organs under different irrigation intervals (I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively). Plants were sprayed by SA and ABA at vegetative and reproductive stages. In both years, the biomass of vegetative organs (leaves and stem), flower production and seed yield per unit area of ajowan decreased with decreasing water availability. All organs mass improved by application of ABA and particularly SA. Essential oil percentage of all organs increased, but essence yield decreased as a result of water limitation. Reduction in essential oil yield of ajowan organs due to water stress strongly related with the reduction of individual organ mass under stress. Foliar spray of ABA and especially SA improved the medicinal and commercial value of ajowan under different irrigation intervals by enhancing plant organs biomass and accumulation of more essential oil.

Keywords: Essence; Flower; Hormone; Organ mass; Seed; Vegetative.

Introduction

Ajowan (*Carum copticum* L.) is a grassy, annual plant of Apiacea family with a white flower and small, brownish seeds which commonly grows in Iran, India, Egypt and Europe. Essential oil of this plant has been reported to have antioxidant (Nickavar and Abolhasani, 2009), anti-cholinergic (Hejazian et al., 2007), anti-histaminic (Boskabady and Shaikhi, 2000) and analgesic effects (Boskabady and Shaikhi, 2006).

Plants growing in natural conditions are constantly exposed to various stresses modifying the intensity or disturbing the course of life processes, causing temporary or irreparable destabilization of the plant organism. The activity of such harmful factors may lead to the inhibition of growth and development of the plants (Shulaev et al., 2008). However, there are reports on the positive effects of limited water supply, as far as the synthesis of secondary metabolites, enzyme activities and solute accumulation are concerned (Singh-Sangwan et al., 2001). For aromatic and medicinal crops, drought may cause significant changes in their metabolites yield and compositions (Bettaieb et al., 2009).

Salicylic acid (SA) is a naturally existing phenolic compound (Raskin, 1992), which has been found to play a key role in the regulation of growth and development such as seed germination, nutrient uptake and transport, photosynthetic rate, flowering and fruit yield under different environmental stresses (Hayat et al., 2010). Considerable interests have focused on SA due to its ability to induce a protective effect on plants under stress, which has been related to its role in nutrient uptake, membrane stability, water relations, stomatal regulation, photosynthesis and inhibition of ethylene biosynthesis (Stevens et al., 2006; Arfan et al., 2007). Exogenous SA affects the ROS generation rate in plants under stress. SA alters the activities of antioxidant enzymes and increases plant tolerance to abiotic stresses (Zhang et al., 2011). Metwally et al. (2003) also found that differential amelioration by SA of stress adaptation and development of damage in plants depends on the plant species and the concentration, method and time of SA application.

Abscisic acid (ABA) is considered an inhibitor of plant growth, while it has a positive effect on plantlet development (Rai et al., 2011; Huang et al., 2012). ABA has been shown to regulate many aspects of plant growth and development in response to environmental stresses such as drought, salinity, cold and pathogen attack (Tsai et al., 1997). Exogenous ABA treatments prior to subjecting plants or tissues to adverse conditions have been reported to improve plant tolerance to osmotic stress (Nayyar and Walia, 2003). It has been also reported that application of exogenous ABA increases drought tolerance of Kentucky bluegrass, improving cell turgor maintenance and reducing damage to cell membranes and photosynthetic systems (Wang et al., 2003). Since the roles of exogenous SA and ABA applications on essential oil content and yield of ajowan organs under water stress are not clear, this work was aimed to investigate these roles in details.

Materials and Methods

Two field experiments were carried out at the Research Farm of the Faculty of Agriculture, University of Tabriz, Tabriz, Iran (Latitude 38° 05′ N, Longitude 46° 17′ E, Altitude 1360 m above sea level) during the growing seasons of 2014 and 2015 to evaluate the effects of salicylic acid (SA) and abscisic acid (ABA) applications on essential oil content and yield of different organs of ajowan (*Carum copticum* L.) under water stress. The soil of the research area was sandy loam with an EC of 0.68 dS m^{-1}, pH of 8.1 and field capacity of 28.8 %. Experiments were arranged as split plot on the bases of randomized complete block design in three replicates. Irrigation treatments (I_1, I_2, I_3 and I_4: irrigation after 70, 100, 130 and 160 mm evaporation as normal irrigation and mild, moderate and severe water stresses, respectively) were located in main plots and foliar sprays of SA (0 and 1 mM) and ABA (0 and 50 µM) were allocated to sub plots. Average maximum and minimum temperatures and rainfall during the experiment in 2014 and 2015 are shown in table 1.

Table 1. Averages of maximum and minimum temperatures and rainfall during the work in 2014 and 2015.

Month	Temperature (°C)		Rainfall (mm)	
	2014	2015	2014	2015
April	23.7	12.6	50.2	43.2
May	29.5	30.45	10.7	1.5
June	37.1	38.9	18	0.9
July	38.9	40.2	1.3	0
August	34.75	31.1	0	26.4
September	20.65	23.45	84.2	24.1

Each plot had 6 rows of 4 m length, spaced 25 cm apart. Seeds of ajowan were treated with Benomyl at a rate of 2 g kg^{-1} before sowing. The seeds were then sown by hand on April 2014 and 2015 at a depth of about 1 cm. All plots were regularly irrigated after sowing until seedling establishment and thereafter irrigations were carried out according to the treatments. Weeds were controlled by hand during crop growth and development as required. Plants were sprayed by water (control), SA and ABA at vegetative and reproductive stages.

Flowers and vegetative organs of 10 plants at flowering stage and 10 other plants at seed maturity from each plot were harvested. All organs (leaves and stems, flowers and seeds) after harvest were dried at room temperature of 20-25 °C to reduce their moisture content to about 10 %. Subsequently, 10 g of different organs of ajowan were hydro-distilled in 500 ml water for 3 hours in a Clevenger apparatus at 250 ºC. The Petroleum ether was used as solvent. The essential oil layer was then removed and stored at 3-5 ºC. Statistical analysis was performed with MSTATC and Excel soft-ware was used to draw the figures. Duncan multiple range test was applied to compare means of each trait at 5 % probability.

Results

Combined analyses of variance showed significant effects of irrigation and hormonal treatments ($P \leq 0.01$) on organ biomass and essence percentage and yield of different organs. Significant differences between years in leaf and stem mass ($P \leq 0.05$), flower yield and essence percentage and yield of all ajowan organs ($P \leq 0.01$) were determined, but seed yield was not significantly affected by year ($P > 0.05$). The interaction of year × irrigation was significant for flower yield, seed essence percentage and flower essence yield ($P \leq 0.01$). Significant interactions of year × hormone for flower yield, essence percentage and yield ($P \leq 0.01$), vegetative mass and essence yield ($P \leq 0.05$), irrigation × hormone for all organs mass, essence yield ($P \leq 0.01$) and essence percentage of vegetative organs ($P \leq 0.05$) and year × irrigation × hormone for vegetative and flower yields and essence yield ($P \leq 0.01$) were also observed.

In both years, mean vegetative yield of ajowan plants decreased with increasing water deficit. Foliar application of hormones under I_1 and I_2 in 2014 and under I_1 in 2015 significantly improved this trait. The highest improvement was recorded for plants treated with salicylic acid (SA). In other irrigation treatments there were no significant differences between hormonal treatments and control (Table 2).

Flower yield of ajowan also decreased with increasing water deficit in both years. Foliar application of SA enhanced flower yield under all irrigation treatments in 2014 and under I_1 in 2015, but ABA treatment only increased flower production under I_1 in 2014 and I_2 and I_3 in 2015. The effect of SA on flower yield was relatively higher than that of ABA (Table 2).

Table 2. Means of vegetative and flower masses and essence yield of ajowan for year × irrigation × hormone.

Year	Treatments		Vegetative mass (g m^{-2})	Flower mass (g m^{-2})	Vegetative essence yield (g m^{-2})	Flower essence yield (g m^{-2})
	Irrigation	Hormone				
2014	I$_1$	Control	256.5 ^{c}	146.4 ^{d}	0.508 $^{e-h}$	3.027 ^{c}
		SA	445.7 ^{a}	280.2 ^{a}	1.016 ^{a}	7.155 ^{a}
		ABA	337 ^{b}	220.8 ^{b}	0.734 ^{c}	5.548 ^{b}
	I$_2$	Control	202 ^{de}	106.3 ^{fg}	0.577 $^{d-g}$	2.282 ^{g}
		SA	354.1 ^{b}	160.0 ^{c}	1.119 ^{a}	4.758 ^{c}
		ABA	216.2 ^{d}	97.4 ^{gh}	0.64 $^{c-e}$	2.425 ^{fg}
	I$_3$	Control	174.8 $^{d-f}$	95.1 ^{hi}	0.545 $^{d-h}$	2.223 ^{gh}
		SA	192.9 $^{d-f}$	113.5 ^{ef}	0.64 $^{c-e}$	3.683 ^{d}
		ABA	177.9 $^{d-f}$	97.6 ^{gh}	0.555 $^{d-g}$	2.689 ^{ef}
	I$_4$	Control	70.53 ^{i}	45.0 ^{k}	0.224 ^{lm}	1.363 ^{jk}
		SA	95.87 ^{hi}	64.4 ^{j}	0.343 $^{j-l}$	2.285 ^{g}
		ABA	80.31 ^{i}	49.6 ^{k}	0.263 $^{k-m}$	1.398 ^{jk}
2015	I$_1$	Control	211.7 ^{d}	137.5 ^{d}	0.417 $^{h-j}$	1.669 ^{ij}
		SA	408.4 ^{a}	216.3 ^{b}	0.885 ^{b}	2.695 ^{ef}
		ABA	284.5 ^{c}	141.9 ^{d}	0.578 $^{d-g}$	1.75 ^{ij}
	I$_2$	Control	204.2 ^{de}	113.9 ^{ef}	0.566 $^{d-g}$	1.464 ^{jk}
		SA	213.5 ^{d}	145.1 ^{d}	0.655 ^{cd}	1.897 ^{hi}
		ABA	212.1 ^{d}	135.9 ^{d}	0.615 $^{c-f}$	1.664 ^{ij}
	I$_3$	Control	129.4 ^{gh}	86.57 ^{i}	0.375 $^{i-k}$	1.218 ^{kl}
		SA	153.5 ^{fg}	121.3 ^{e}	0.468 $^{g-j}$	1.541 $^{i-k}$
		ABA	165.1 $^{e-g}$	120.5 ^{e}	0.496 $^{f-i}$	1.527 $^{i-k}$
	I$_4$	Control	57.05 ^{i}	47.1 ^{k}	0.181 ^{m}	0.738 ^{m}
		SA	79.5 ^{i}	49.7 ^{k}	0.286 $^{k-m}$	0.887 ^{lm}
		ABA	68.28 ^{i}	44.4 ^{k}	0.226 ^{lm}	0.713 ^{m}

Different letters in each column indicate significant difference at $P \leq 0.05$ (Duncan multiple range test).
I$_1$, I$_2$, I$_3$, I$_4$: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Seed yield of hormonal treated and none-treated plants significantly decreased as water availability decreased. Foliar application of hormones, particularly SA improved seed yield of ajowan under all irrigation treatments. However, this improvement was more evident well watering (Figure 1).

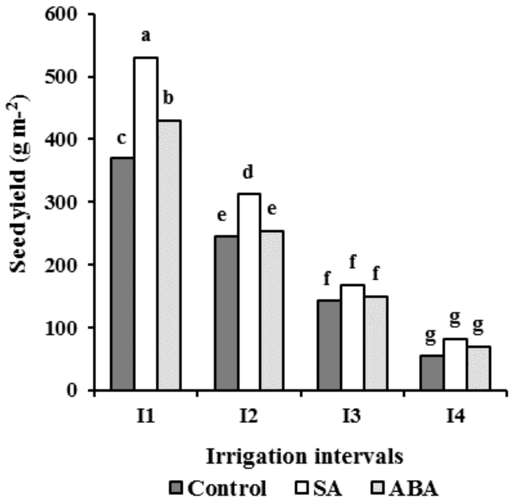

Figure 1. Mean seed yield of ajowan affected by hormonal treatments under different irrigation intervals. Different letters indicate significant difference at P ≤ 0.05 (Duncan multiple range test).
I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Essence percentage of vegetative organs in 2014 was higher than that in 2015 (Figure 2A). Mean essence percentage of vegetative organs in hormonal treated and none-treated plants increased (Figure 2B), but mean essence yield decreased as water stress increased (Table 2). The highest improvement in essence percentage was achieved by application of SA (Figure 2B). Essence yield of vegetative organs was improved by application of SA under I_1 and I_2 in 2014 and under I_1 in 2015 and by ABA treatment under I_1 in both years (Table 2).

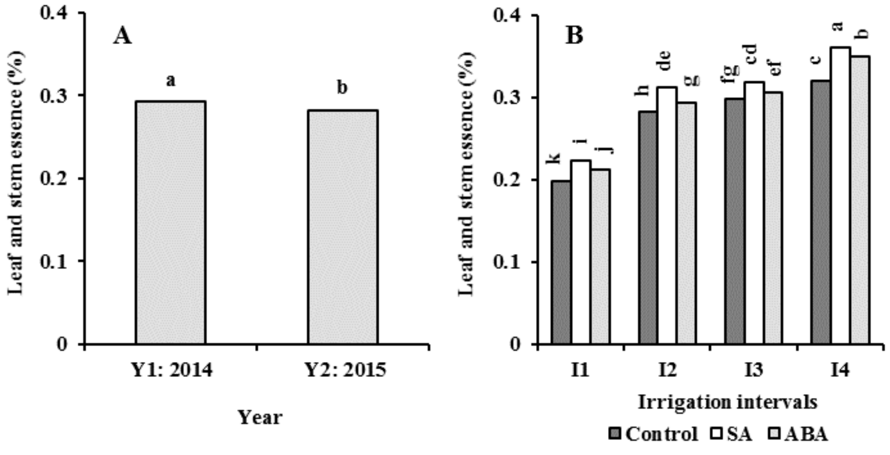

Figure 2. Mean essence percentage of vegetative organs of ajowan for years (A) and also for hormonal treatments under different irrigation intervals (B).
Different letters indicate significant difference at P ≤ 0.05 (Duncan multiple range test).
I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Flower essence percentage increased (Figure 3A), but essence yield decreased (Table 2) with increasing irrigation intervals. However, differences between I_1 and I_2 and also between I_2 and I_3 in essence percentage were not significant (Figure 3A). The flower essence content of all plants in the first year was higher than that in the second year. In the first year, SA treated plants had significantly higher flower essence than ABA treated and control plants, while in the second year, there was no significant difference between hormonal treated and control plants (Figure 3B). Plants treated with SA and ABA were superior in flower essence yield under all irrigation intervals in both years, but this superiority decreased with decreasing water availability (Table 2).

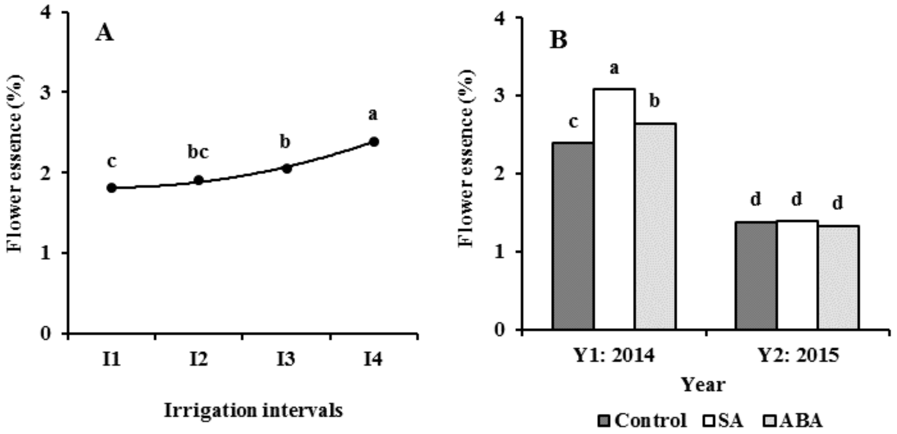

Figure 3. Mean flower essence percentage of ajowan for irrigation intervals (A) and also for hormonal treatment in two years (B).
Different letters indicate significant difference at $P \leq 0.05$ (Duncan multiple range test).
I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

The highest seed essence percentage was obtained from SA treated plants, followed by ABA treated and control plants, respectively (Figure 4A). In the first year, seed essence percentage enhanced as water deficit increased, but in the second year it was only increased under I_3 and I_4 (Figure 4B).

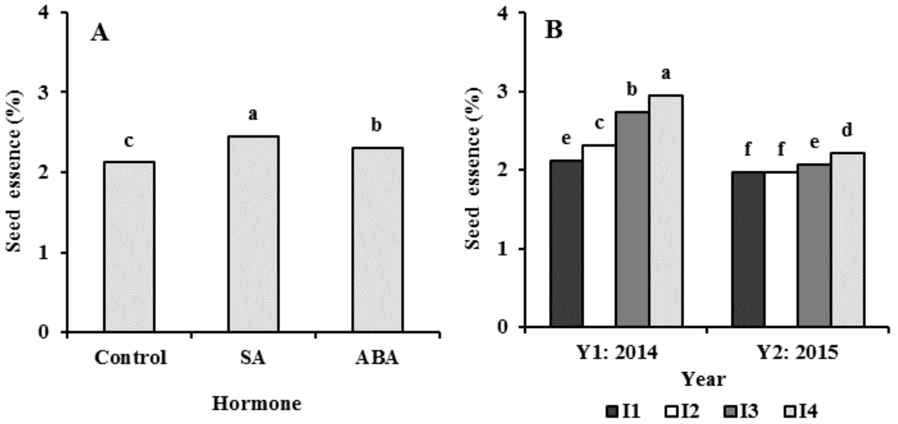

Figure 4. Mean seed essence percentage of ajowan for hormonal treatments (A) and also for irrigation intervals in two years (B).
Different letters indicate significant difference at $P \leq 0.05$ (Duncan multiple range test).
I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Essence yield of ajowan seeds in 2014 was higher than that in 2015 (Figure 5A). Seed essence yield of all plants decreased as water stress increased. The highest seed essence yield was obtained from SA treated plants, followed by ABA treated and control plants under all irrigation treatments. However this superiority of SA treated plants diminished with declining water supply (Figure 5B).

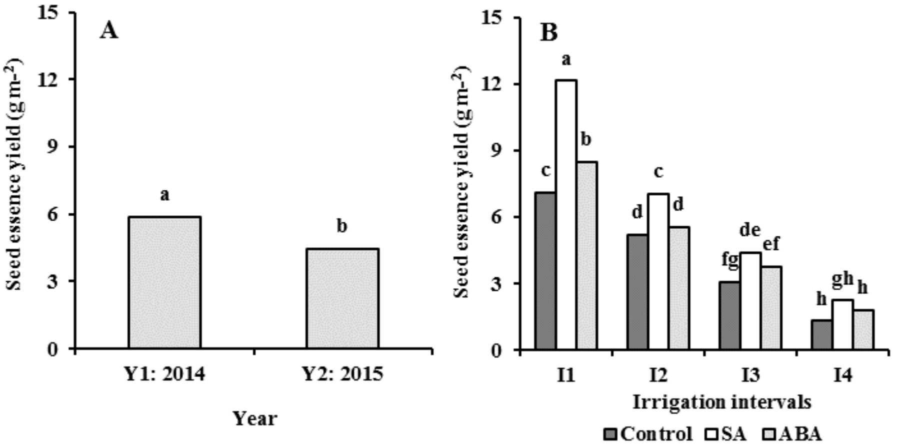

Figure 5. Mean seed essence yield of ajowan for years (A) and also for hormonal treatments under different irrigation intervals (B).
Different letters indicate significant difference at $P \leq 0.05$ (Duncan multiple range test).
I_1, I_2, I_3, I_4: irrigation after 70, 100, 130 and 160 mm evaporation, respectively.

Discussion

Decreasing yield per unit area of vegetative organs (leaves and stem) in ajowan due to water stress (Table 2) was clearly resulted from declining dry matter accumulation and plant growth rate during vegetative stages (Ghassemi-Golezani et al., 2009). Leaf and stem growth rate are very sensitive to water stress because they are dependent on cell expansion (Hearn, 1994). Water availability affect the growth and physiological processes of all plants, since water is the primary component of actively growing plants ranging from 70-90 % of plant fresh mass (Gardner and Gardner, 1984). The reductions in plant dry weight under water stress was perhaps due to the decline in the cell enlargement and more leaf senescence resulting from reduced turgor pressure (Shao et al., 2008).

Decreased flower production of ajowan is a reflection of decreased plant size as a result of water deficit (Table 2). Reduction in yield of vegetative organs and especially flower yield due to water limitation (Table 2) led to considerable deduction in seed yield per unit area (Figure 1). Seed production per unit area is more closely related with flower yield (Heitholt, 1995). Amelioration of water deficit through the use of plant growth regulating substances has been suggested as a potential solution to water stress (Ghassemi-Golezani et al., 2015a).

Improving production of all organs of ajowan by application of ABA and particularly SA (Table 2, Figure 1) may be related with regulating effects of these hormones on plant growth and development. Foliar application of SA enhances chlorophyll content, relative water content, leaf area index (Ghassemi-Golezani and Hosseinzadeh-Mahootchi, 2015), maximum quantum yield of photosystem II (Ghassemi-Golezani and Lotfi, 2015), stomatal conductance, root and shoot dry mass production and consequently seed yield (Ghassemi-Golezani et al., 2015b). SA also stimulates flowering (Cleland and Ajami, 1974). Genetic studies pointed out to SA as a regulator of flowering time that interacts with both the photoperiod-dependent and autonomous pathways (Martinez et al., 2004). It appears that SA might be necessary, but not sufficient to induce flowering (Wada et al., 2010). Thus, the beneficial effect of SA on seed yield may be due to increasing flower yield and translocation of more photo assimilates to seeds during seed filling (Lotfi and Ghassemi-Golezani, 2015).

It has been reported that the role of ABA in closing stomata of drought stressed plants is vital for fast growth resumption and recovery of water content of plants (Zhang et al., 2006). This suggests that foliar spray of ABA is favouring vegetative growth of plants as shown for ajowan (Table 2) and soybean (Travaglia et al., 2009). Exogenous ABA treatment may promote floral initiation by down-regulating ethylene synthesis (Sharp et al., 2009), thereby enhancing flowers (Table 2) and seeds. ABA application also improves source to sink transport of assimilates during seed filling phase (Yang et al., 2004) that can enhance seed size and yield (Figure 1).

The high essential oil percentage of ajowan vegetative organs (Figure 2A) and flowers (Figure 3B) in 2014 may be caused by high temperature and no rainfall before harvesting in August, compared with 2015 (Table 1). In drought stressed plants more metabolites were produced, so the essential oil percentage in all organs of ajowan increased (Figures 2B, 3A, 4B). It was reported that water stress increases essential oil accumulation via a higher density of oil glands due to the reduction in leaf area (Simon et al., 1992). In addition, the plants produce high terpenes under water stress due to a low allocation of carbon to the growth, suggesting a trade-off between growth and defence (Turtola et al., 2003). High essential oil content under drought stress also reported in dill (Ghassemi-Golezani et al., 2015c; Ghassemi-Golezani et al., 2008) and balm (Aliabadi et al., 2009). Opposing results, however, indicated that water limitation either resulted in lower essential oil accumulation (Figueiredo et al., 2008) or had no effect on essential oil content (Khazaie et al., 2008).

Improvement in essential oil percentage by foliar application of hormones especially with SA (Figures 2B, 3B, 4A) might be due to the increase in cell growth, nutrients uptake or changes in leaf oil gland population and monoterpenes biosynthesis (Idrees et al., 2010). Improvement of essential oil percentage in response to SA application has been also reported for other plant species (Gharib, 2006; Bahreininejad et al., 2013). Exogenous ABA might enhance the production of carbon based secondary metabolites (Ibrahim and Jaafar, 2013).

Reduction in essential oil yield of ajowan organs under water stress strongly associated with the reduction of individual organ mass under stress (Table 2, Figure 5B) as also reported for dill under salt stress (Ghassemi-Golezani et al., 2011). The high seed essence yield of ajowan in 2014 (Figure 5A) can be attributed to high essence percentage (Figure 4B) and more rainfall during seed filling, compared with 2015 (Table 1). Ghassemi-Golezani et al. (2015c) showed that the highest essence percentage of dill was recorded under drought stress, but the highest essence yield was obtained under well watering. This reduction is due to disturbance in photosynthesis and carbohydrate production and suppression of the plant growth and yield under stress condition (Flexas and Medrano, 2002).

Foliar spray of ABA and particularly SA improved the medicinal and commercial value of ajowan under different irrigation intervals by enhancing plant biomass and accumulation of secondary metabolites (Table 2, Figure 5B). SA may also induce the expression of many defence genes which encode particular enzymes of secondary metabolic pathway to form bioactive compounds such as phenolics (Ali et al., 2007). ABA induces the synthesis of several bioactive compounds and increases the secondary metabolites which will help to improve the medicinal potential of plants (Awate and Gaikwad, 2014). ABA can also induce the expression of antioxidant genes and enhance the capacity of antioxidant defence systems, including enzymatic and non-enzymatic constituents (Jiang and Zhang, 2001).

Conclusions

The biomass of all ajowan organs per unit area decreased with increasing water stress. However, all organs mass under different irrigation intervals improved by foliar application of ABA and particularly SA. Although essential oil percentage of all organs increased with decreasing water availability, essence yield per unit area decreased as a result of reduction in individual organ mass under stressful conditions. Foliar spray of ABA and especially SA improved essence production of ajowan under different irrigation intervals by enhancing plant organs biomass and essential oil percentage. Therefore, exogenous application of SA could be a better treatment to promote growth and essence accumulation in ajowan organs under favourable and unfavourable environmental conditions.

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