



## Evaluation of some morphological traits and oil content of coriander seeds in response to bio-fertilizer and salicylic acid under water stress

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### Abstract

Water stress is one of the most environmental factors affecting and limiting plant growth and productivity, mainly in arid and semi-arid regions in the world. This research was undertaken in 2014 to evaluation of changes in leaves and nodes per plant, root/shoot ratio and oil content of coriander (*Coriandrum sativum* L.) seeds in response to biological and chemical fertilizers, and salicylic acid under different irrigation treatments in Kerman shah province, Iran. The experiment was arranged as split-plot factorial based on randomized complete block (RCB) design with three replications. Treatments were 3 levels of water supply (irrigation after 60, 90 and 120 mm evaporation from class A pan) and 4 levels of fertilization (control, 100 kg $\text{ha}^{-1}$  Urea, Nitrokara and biofertilizer, and 50% Urea + Nitrokara) and foliar application of salicylic acid (0 and 1 mM). Increasing irrigation intervals led to significant reduction of leaves and nodes per plant, shoot dry weight, and oil content and yield of coriander seeds. However, root/shoot ratio increased as a result of water deficit. Application of nitrogen fertilizers, particularly 50% Urea and Nitrokara, had a positive and additive effect on number of leaves and nodes per plants, shoot dry weight, root/shoot ratio, and seed oil percentage and yield. On the other hand, plants treated with salicylic acid had higher shoot dry weight and seed oil content and yield compared with untreated plants. Therefore, the combined utilization of bio-fertilizer and salicylic acid can be recommended to improve the oil content and yield of coriander seeds under different water conditions.

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## Introduction

Coriander (*Coriandrum sativum* L.) is an annual herb which possesses nutritional and medicinal properties, and also it is one of the most commonly used spices (Leena *et al.*, 2012). It is a multipurpose herb grown mainly for its foliage and seeds (Burdock and Carabin, 2009). Coriander fruits (seeds) contain between 10 to 27.7% fatty acids and up to 2.6% essential oils, which may be used for many industrial purposes (Diederichsen, 1996). Since the coriander seeds have strong and typical scent, they are appreciated worldwide as basic ingredients of many traditional foods, particularly curry powder (Mahendra and Bisht, 2011; Sahib *et al.*, 2013).

Drought stress occurs when water intake is less than transpiration. This is possible due to excessive water loss, absorption reduction or both of them in the plants. Turgor reduction is one of the water deficit signs; therefore decreasing cell and plant growth (Baigorri *et al.*, 1999). A common negative effect of water stress on plants is the reduction of dry biomass (Zhao *et al.*, 2006). The modification in root/shoot ratio or temporary storage of reserves in stem is another effect of water deficit. Root/shoot ratio helps to assess the overall health of plants and is used to evaluate the stress avoidance potential of plants (Bush, 1995).

Salicylic acid (SA) is a phenolic compound and natural constituent of plant (Raskin, 1992). Exogenous application of SA influences a wide variety of plant processes, including stomatal regulation and photosynthesis, chlorophyll content and ion uptake (Ananieva *et al.*, 2002). Action mechanism of salicylic acid against stress returns to its role in antioxidant enzymes regulation and compounds containing active oxygen species in plant (Raskin, 1992). Role of SA at a certain level in moderate and severe abiotic stress may be different that could be attributed to redox regulations in plant cells. The effectual level of SA for roots may be different from the one for shoots, and the antioxidant enzymes in roots may be insensitive to SA (Yuan and Lin, 2008). Studies have shown that the exogenous application of SA counteracts the inhibiting effects of drought stress on plant growth in different crop species (Kadioglu *et al.*, 2011).

While application of chemical nitrogen fertilizers can supply sufficient nutrients to improve plant production, they are also responsible for water, air and soil pollutions and can spread cancer causing agents (Mishra *et al.*, 2013). The utilization of biological nitrogen fixation (BNF) technology can decrease the use of urea, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent (Choudhury and Kennedy, 2004). Bio-fertilizer as essential components of organic farming, play a vital role in maintaining long term fertility and sustainability of soil (Mishra *et al.*, 2013) and help in reducing the application of chemical fertilizers in sustainable agriculture. In addition to atmospheric nitrogen fixation and phosphorous solubilisation, bio-fertilizers also help in stimulating the plant growth hormones, providing better nutrient uptake and increasing plant tolerance against drought stress (Anandaraj and Delapierre, 2010). Crop yield and growth increase 20 to 30% by replacing chemical nitrogen and phosphorus with bio-fertilizers (Vessey, 2003).

According to Yeganehpour *et al.* (2016), combined application of 50% Urea, Nitrokara and salicylic acid had the highest effect on leaf area, chlorophyll content and seed yield of coriander under favorable and limited irrigation conditions.

Therefore, this research was carried out to detect the effects of bio-fertilizer and salicylic acid on number of leaves and nodes per plant, shoot dry weight, root/shoot ratio and oil content of coriander under different irrigation treatments.

## Materials and methods

### *Field conditions and experimental design*

A field experiment was conducted as split-plot factorial based on randomized complete block design with three replications in 2014 at the Research Farm of Kermanshah, Iran (latitude 47°34'N, longitude 34°39'E, altitude 1200m above sea level) to evaluate the effects of biological and chemical nitrogen fertilizers, salicylic acid and different irrigation intervals on some traits of coriander.

The climate of research area is characterized by mean annual precipitation of 350.5 mm, mean annual temperature of 10°C, mean annual maximum

temperature of 17°C and mean annual minimum temperature of 4.5°C. The soil was loamy with field capacity of 28.4%. Soil test results are shown in Table 1.

**Table 1.** Physical and chemical characteristics of research field soil.

Soil	Sand (%)	Clay (%)	Silt (%)	EC (dS m <sup>-1</sup> )	pH	OC (%)	Fe (ppm)	K (mg kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	N (%)
Loamy	29	26	45	0.4	8.09	2	1.38	232	14.1	0.2

#### Treatments

Irrigation treatments (I<sub>1</sub>, I<sub>2</sub> and I<sub>3</sub>: irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively) were located in main plots and combination of fertilization (control, 100 kg ha<sup>-1</sup> Urea, Nitrokara (as bio-fertilizer), and 50% Urea + Nitrokara) and salicylic acid (0 and 1 mM) were allocated to sub plots.

Coriander seeds were inoculated with Nitrokara, a bio-fertilizer containing *Azorhizobium caulinodans* bacteria with formulation of Kara technique living industrial company, and sown by hand on May 2<sup>nd</sup> 2014 with a density of 40 seeds per m<sup>2</sup>. Urea was applied on the basis of soil test (1/3 at sowing date, 1/3 after thinning and 1/3 at vegetative stage).

All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. The plants were sprayed twice with salicylic acid (0 and 1 mM) at stages of stem elongation and flowering using a hand pump sprayer at the time of 07:00 to 08:00 AM, until both sides of the leaves completely became wet. Weeds were controlled by hand during plant growth and development as required. Each plot had 6 rows of 4m length, spaced 20 cm apart.

#### Measurements

At the time of full flowering of coriander, 10 plants were randomly selected from the central rows of each plot and the average number of leaves and nodes per plant were recorded. At the maturity, 10 selected plants were carefully harvested and roots were washed with distilled water and separated from the aerial organs and dried in an oven with 80°C for 48 hours.

Then dry weights of root and shoot were determined with a digital scale and root to shoot ratio was calculated.

At the physiological maturity, plants of 1 m<sup>2</sup> in the middle part of each plot were harvested and grain yield per unit area was recorded. In order to determine the seeds oil, 10g of seed samples were ground and oil percentage was determined using soxhlet apparatus and subsequently oil yield per unit area was calculated.

#### Statistical analysis

Data were analyzed by SAS 9.1.3 software and comparison of means was performed using the Duncan's multiple range tests at  $p \leq 0.05$ . Excel software was used to draw the Figs.

## Results and discussion

### Number of leaves and nodes per plant

Leaves and nodes per plant significantly influenced by irrigation intervals, nitrogen fertilization and their interaction, but the effect of salicylic acid were not significant on these traits (Table 2).

In all fertilization treatments, decreasing water supply led to reduction in leaves per plant. Although, the highest number of leaves per plant was obtained with application of 50% Urea and Nitrokara under well watering, additive effect of nitrogen fertilization was remarkable under water limitation.

So that in plants irrigated with 60 mm (I<sub>1</sub>), 90 mm (I<sub>2</sub>) and 120 mm evaporation intervals (I<sub>3</sub>), application of 50% Urea and Nitrokara led to 15%, 16.7% and 20% increase in leaves number, compared to no-fertilization (F<sub>0</sub>) under same irrigation treatments, respectively.

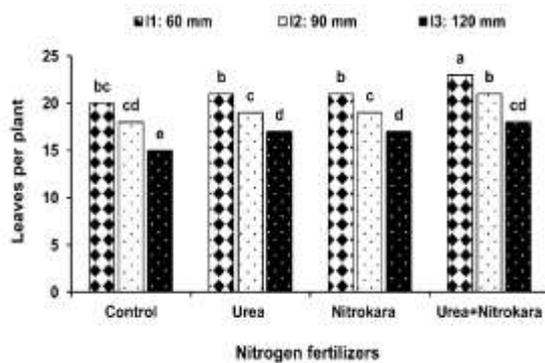
However, there was no significant difference between plants treated with Urea (F<sub>1</sub>) and

Nitrokara (F<sub>2</sub>) in terms of leaves number in none of the irrigation treatments (Fig. 1).

**Table 2.** Analysis of variance of effect of irrigation, fertilization and salicylic acid on some traits of coriander.

Sources of variation	df	Mean Squares					
		Leaves per plant	Nodes per plant	Dry weight of shoot	Root/Shoot	Oil content	Oil yield
Replication	2	15.83 <sup>ns</sup>	0.29 <sup>ns</sup>	28.16 <sup>ns</sup>	0.96 <sup>ns</sup>	0.8821 <sup>**</sup>	15.94 <sup>ns</sup>
Irrigation (I)	2	403.17 <sup>**</sup>	7.15 <sup>**</sup>	931.37 <sup>**</sup>	28.31 <sup>**</sup>	12.8 <sup>**</sup>	1239.8 <sup>**</sup>
E <sub>a</sub>	4	39.20	1.09	53.88	2.13	0.03	316.2
Fertilizer (F)	3	308.39 <sup>**</sup>	4.44 <sup>**</sup>	883.05 <sup>**</sup>	19.26 <sup>**</sup>	16.88 <sup>**</sup>	1803.2 <sup>**</sup>
I × F	6	205.11 <sup>**</sup>	2.91 <sup>**</sup>	802.15 <sup>**</sup>	0.815 <sup>ns</sup>	9.9 <sup>**</sup>	1453.5 <sup>**</sup>
Salicylic acid (SA)	1	11.03 <sup>ns</sup>	0.87 <sup>ns</sup>	716.67 <sup>**</sup>	0.702 <sup>ns</sup>	12.82 <sup>**</sup>	1075.9 <sup>**</sup>
I × SA	2	5.18 <sup>ns</sup>	0.61 <sup>ns</sup>	639.33 <sup>**</sup>	0.06 <sup>ns</sup>	10.17 <sup>**</sup>	1137.9 <sup>**</sup>
F × SA	3	2.94 <sup>ns</sup>	0.32 <sup>ns</sup>	561.96 <sup>**</sup>	0.04 <sup>ns</sup>	8.1 <sup>**</sup>	1308.1 <sup>**</sup>
I × F × SA	6	0.66 <sup>ns</sup>	0.15 <sup>ns</sup>	440.29 <sup>**</sup>	0.01 <sup>ns</sup>	0.0001 <sup>ns</sup>	10.16 <sup>ns</sup>
E <sub>b</sub>	42	13.09	0.85	20.62	0.835	0.0004	13.96
C.V.(%)	--	9.33	9.64	10.35	11.76	10.2	11.5

<sup>ns</sup> and <sup>\*\*</sup>: No significant and significant at p ≤ 0.01, respectively.

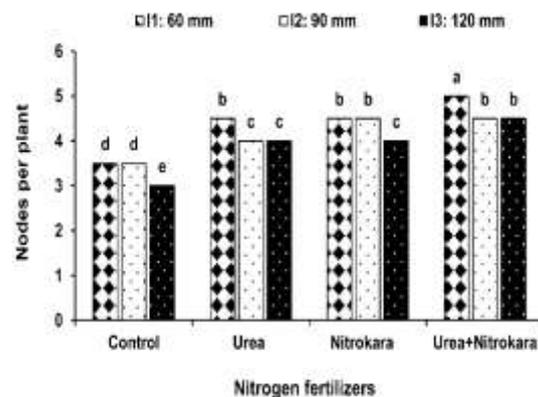


**Fig. 1.** Means of coriander leaves number affected by irrigation × fertilization. Different letters indicate significant difference at p ≤ 0.05 (Duncan test).

The reduction of leaves number under the limited soil moisture available (Fig. 1) is one of the drought tolerance mechanisms to avoid the excessive transpiration or water conservation strategy (Jones, 1992). Reduction effects of water stress on the leaves per plant have been also reported for borage (Dastborhan *et al.*, 2013) and marigold (Riaz *et al.*, 2013). The significant increment in number of leaves as a result of combined application of 50% urea and Nitrokara can be attributed to the enhancing essential nutrients uptake and production of plant growth promoting hormones in this conditions. Nitrogen has an important role in both cells division and elongation. Furthermore, nitrogen may accelerate the decomposition of soil organic matter.

Hence, an increase might be occurred in water and nutrients holding capacity, plant root systems activity and improving soil structure.

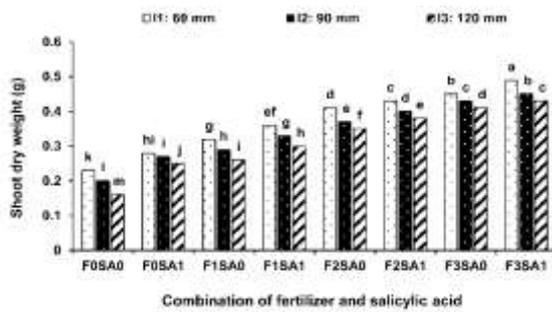
Changes in the nodes number with application of nitrogen fertilizers was higher than leaves number under different irrigation treatments. Nodes per plant were minimal in untreated plant under all irrigation intervals, but it was increased with application of different nitrogen fertilizer, especially 50% Urea + Nitrokara, particularly under severe water stress. There was no significant difference between I<sub>2</sub> and I<sub>3</sub> treatments in plants treated with Urea and 50% Urea + Nitrokara (Fig. 2).



**Fig. 2.** Means of coriander nodes number affected by irrigation × fertilization. Different letters indicate significant difference at p ≤ 0.05 (Duncan test).

*Dry weight of shoot*

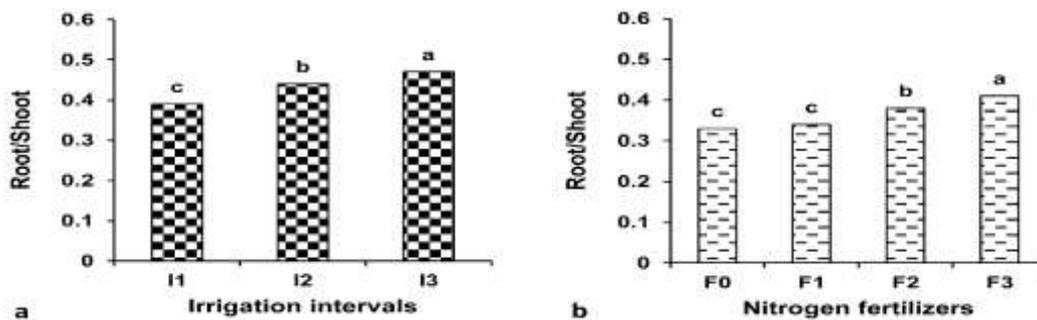
Results of analysis of variance showed that effects of irrigation intervals, fertilization and SA application were significant for shoot dry weight of coriander. This trait was also significantly affected by interactions of irrigation × fertilizer, irrigation × SA, fertilizer × SA and irrigation × fertilizer × SA (Table 2). Dry weight of shoot significantly decreased with increasing irrigation intervals under different fertilization and hormonal treatments. Maximum shoot dry weight under all irrigation treatments was related to application of 50% Urea + Nitrokara + SA. However these changes were considerable under severe water deficit. So that in the plants irrigated with 60 mm (I<sub>1</sub>), 90 mm (I<sub>2</sub>) and 120 mm evaporation intervals (I<sub>3</sub>), combination of SA, 50% Urea and Nitrokara led to 113%, 125% and 168.7% increase in shoot dry weight, compared with control (F<sub>0</sub>SA<sub>0</sub>), under same irrigation treatments, respectively (Fig. 3).



**Fig. 3.** Changes in dry weight of coriander shoot affected by Irrigation × fertilization × SA.

Different letters indicate significant difference at  $p \leq 0.05$  (Duncan test).

F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>: No fertilizer (control), Urea, Nitrokara (bio-fertilizer), and 50% Urea+Nitrokara, respectively SA<sub>0</sub> and SA<sub>1</sub>: Non-application of salicylic acid and application of 1 mM salicylic acid, respectively.



**Fig. 4.** Means of root/shoot of coriander affected by irrigation intervals (a) and fertilization (b).

Different letters indicate significant difference at  $p \leq 0.05$  (Duncan test).

I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively, F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>: No fertilizer (control), Urea, Nitrokara (bio-fertilizer), and 50% Urea +Nitrokara, respectively.

Water stress reduced biomass of coriander plants due to the reduction of leaf area and chlorophyll content under this conditions (Yeganehpour *et al.*, 2016). Bio-fertilizers and nitrogen fertilizers are activator of some enzymatic systems and can influence plants growth and production.

The profitable effects of bio-fertilizers on development of the plant aerial parts are mainly attributed to root development, higher and better uptake of water and mineral by roots, synthesis of growth promoting substances and atmospheric nitrogen fixation (Okon and Itzigsohn, 1995). High shoot dry weight in plants treated with salicylic acid is directly related to enhanced chlorophyll content and LAI under favorable and stressful conditions (Yeganehpour *et al.*, 2016).

*Root/Shoot ratio*

Water deficit and nitrogen fertilization had a significant effect on root/shoot ratio in coriander, but there was no significant difference between plants treated with salicylic acid and untreated plants in root/shoot ratio (Table 2).

Root/shoot ratio was significantly improved under water limitation. Plant irrigation after 90 and 120 mm evaporation led to 12.8% and 20.5% increment in root/shoot ratio, compared with well watering, respectively (Fig. 4a). Application of nitrogen fertilizers increased root/shoot ratio by 3-24.2%, compared with control (F<sub>0</sub>). The lowest and the highest root/shoot ratio were recorded for untreated plants (F<sub>0</sub>) and plants treated with 50% Urea + Nitrokara (F<sub>3</sub>), respectively. However, there was no significant difference between F<sub>0</sub> and F<sub>1</sub> treatments (Fig. 4b).

Plants take water and minerals through roots to sustain life and compete for nutrition. When plants are grown under stress, the competition is more pronounced (Riaz *et al.*, 2010) and roots determine the tolerance of plants against stress under such conditions (Riaz *et al.*, 2013). Higher root growth under water deficit conditions can increase drought tolerance in plants (Chaves and Oliveria, 2004). Plants are able to modify their growth and to adjust allocation patterns between the organs in response to environmental changes. Increment in the root/shoot ratio can be due to different sensitivities of the root and shoot to endogenous ABA, or to a greater osmotic adjustment in root compared with shoot (Samarah *et al.*, 2007) under stress conditions. Plants respond to drought by increasing the proportion of assimilate diverted to growth and thus, increase the shoot/root ratio and the volume of available water for plant (Riaz *et al.*, 2013). Under water limitation, leaf growth is limited by the supply of nutrients and water, and less assimilates are incorporated above-ground. The excess assimilates are then transported to the root, enhancing root growth relative to that of shoots (Grechi *et al.*, 2007).

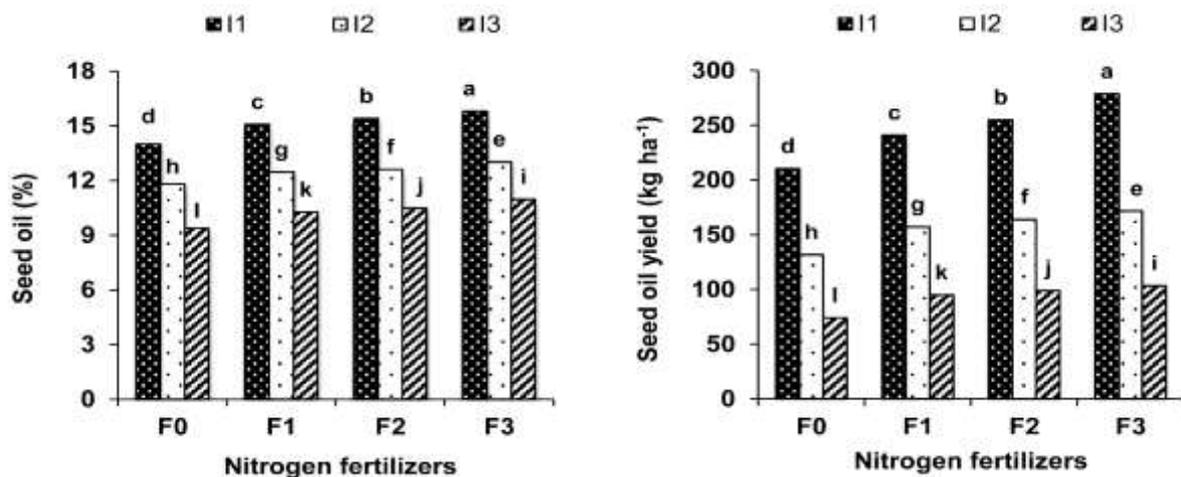
*Seed oil percentage and yield*

Oil content and yield of coriander seeds were significantly affected by irrigation intervals, nitrogen

fertilization and foliar application of salicylic acid. Interactions of irrigation × fertilizer, irrigation × SA and fertilizer × SA were also significant for this trait (Table 2). Changes of oil content in response to the treatments were lower than oil yield. Oil content and yield of coriander seeds decreased with reduction in water availability. Application of nitrogen fertilizers, especially 50% Urea and Nitrokara, improved seed oil percentage and yield under all irrigation treatments. This increment was 12.8%, 10.3% and 17% for oil content and 32.5%, 30.3% and 39.7% for oil yield in plants irrigated with 60mm, 90 mm and 120 mm evaporation intervals, respectively (Fig. 5).

Improvement in seed oil content and yield of coriander was also observed by foliar application of SA. The highest oil percent (15.6%) and oil yield (265.4 kg ha<sup>-1</sup>) were related to plants treated with SA under well watering. However, additive effect of SA application under severe water deficit (I<sub>3</sub>) was remarkable on these traits (Fig. 6). Seed oil content and yield of coriander was also increased with application of SA under different fertilizations.

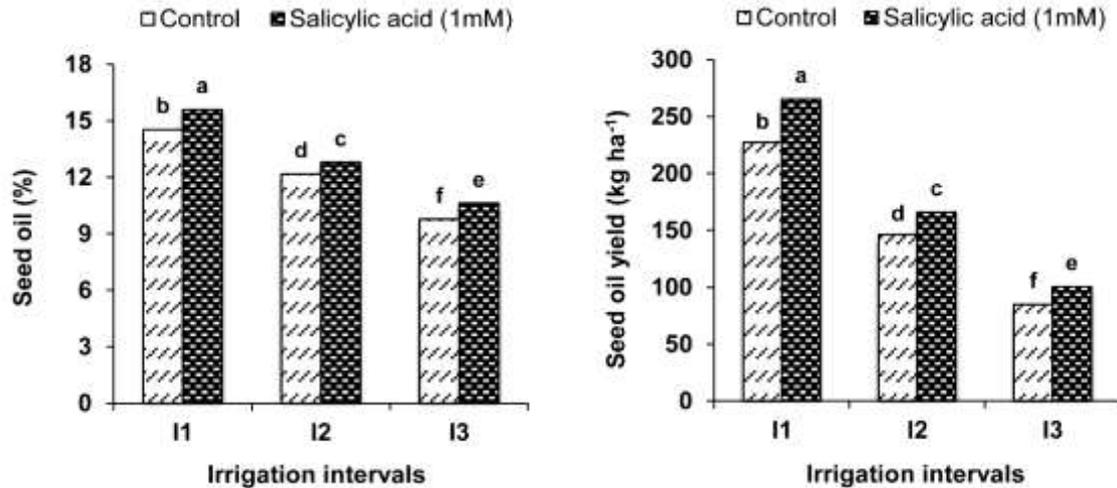
The highest increment in oil content and yield by foliar application of salicylic acid was recorded for plants treated with 50% Urea and Nitrokara (Fig. 7).



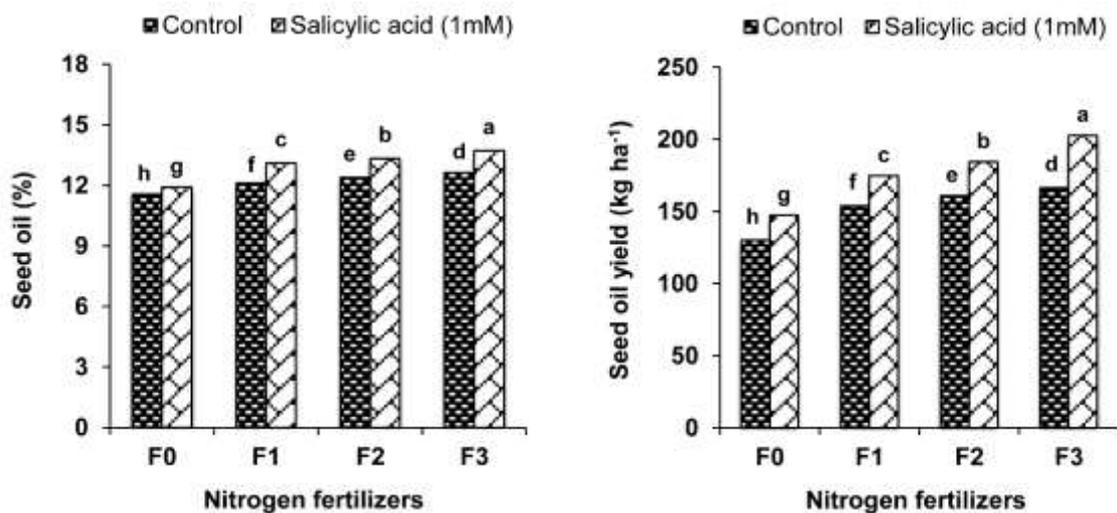
**Fig. 5.** Means of oil percentage and yield of coriander seeds affected by irrigation × fertilization.

Different letters indicate significant difference at p ≤ 0.05 (Duncan test).

I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively. F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>: No fertilizer (control), Urea, Nitrokara (bio-fertilizer), and 50% Urea +Nitrokara, respectively.



**Fig. 6.** Means of oil content and yield of coriander seeds affected by irrigation × salicylic acid. Different letters indicate significant difference at  $p \leq 0.05$  (Duncan test). I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively.



**Fig. 7.** Means of oil content and yield of coriander seeds affected by fertilization × salicylic acid. Different letters indicate significant difference at  $p \leq 0.05$  (Duncan test). F<sub>0</sub>, F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>: No fertilizer (control), Urea, Nitrokara (bio-fertilizer), and 50% Urea +Nitrokara, respectively.

The decrease in seed oil content under water stress is a common phenomenon (Ali *et al.*, 2009). Reduction in oil yield due to water deficit is related to changes of seed oil percentage (Fig. 5) and strongly seed yield per unit area (Yeganehpour *et al.*, 2016) under different water supply. Salicylic acid naturally is produced in plants in very low amounts. It appears as a signal molecule or chemical messenger and its role in defense mechanism has been well established in plants (Gunes *et al.*, 2007). Salicylic acid can regulate various plant metabolic processes and maintain

plant-nutrient status hence, to protect plants under abiotic stress conditions (Khan *et al.*, 2015). Beneficial effects of salicylic acid on seed oil percentage and yield of coriander under stressful condition (Fig. 6 and 7) may be related with enhancing essential nutrients uptake (Mady, 2009), detoxifying super oxide radicals (Joseph *et al.*, 2010), reducing lipid peroxidation (Aftab *et al.*, 2011), improving photosynthetic pigments and consequently enhancing photosynthesis and growth (Ullah *et al.*, 2012; Yeganehpour *et al.*, 2016).

## Conclusions

Reduction of water availability decreased the number of leaves per plant, nodes per plant, plant biomass, and oil content and yield of coriander seeds and increased root/shoot ratio. Application of nitrogen fertilizers, particularly 50% Urea and Nitrokara, had a positive and additive effect on number of leaves and nodes per plant, shoot dry weight, root/shoot ratio and oil yield. On the other hand, plants treated with salicylic acid had higher shoot dry weight and oil content and yield compared with untreated plants. Therefore, the combined utilization of bio-fertilizer and salicylic acid can be recommended to improve the oil content and yield of coriander seeds under different water conditions.

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