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# The impact of Nitrokara and salicylic acid on proline content and essential oil composition of coriander under different water supply

**Farhood Yeganehpoor, Saeid Zehtab-Salmasi, Kazem Ghassemi-Golezani, Jalil Shafagh-Kolvanagh and Soheila Dastborhan**

**Abstract**
Water stress causes many physiological and biochemical changes in plants. Salicylic acid and bio-fertilizers may enhance the plant tolerance to environmental stresses. Thus, two field experiments were arranged as split-plot factorial based on RCB design with three replications in 2014 and 2015 to evaluate the effects of chemical and biological nitrogen fertilizers and salicylic acid on yield, proline and essential oil contents and essential oil composition of coriander seeds under different irrigation treatments in Kermanshah province, Iran. Treatments were 3 levels of water supply (irrigation after 60, 90 and 120 mm evaporation from class A pan), 4 levels of nitrogen fertilizer (control,100 $kgha^{-1}$ urea, Nitrokara (as bio-fertilizer) and 50% urea + Nitrokara) and foliar application of salicylic acid (0 and 1 mM). Irrigation treatments and combination of nitrogen fertilizer and salicylic acid were located in the main and sub plots, respectively. Water deficit caused a significant reduction in seed and essential oil yields and a significant increment in leaf proline and seed essential oil contents. Application of salicylic acid and nitrogen fertilizers, especially 50% urea and Nitrokara, hadanadditiveeffecton content of proline and essential oil, and yield of seed and essential oil under all irrigation treatments. Chemical compositions of essential oil of coriander seeds were determined by gas chromatography mass spectroscopy (GC/MS) and forty two components were identified. The main constituent of seed essential oil under all treatments was Linalool. Decreasing water availability led to a slight increment in concentration of all major compounds of essential oil, except γ-terpinene. Application of SA and nitrogen fertilizers (especially 50% urea + Nitrokara) improved essential oil quality by increasing the percentage of linalool and γ-terpinene. Therefore, salicylic acid treatment alleviates the negative effects of water stress and can be recommended for improving field performance of coriander under different water availabilities when applied with bio-fertilizers.

**Keywords:** Coriander, Bio-fertilizer, Essential oil components, Salicylic acid, Water stress

## 1. Introduction
Coriander (*Coriandrum sativum* L.) belonging to the Apiaceae family is an annual herb which is called Geshniz in Persian. It is one of the most commonly used spices and possesses nutritional and medicinal properties [1]. All parts of the coriander plant are edible, but the fresh leaves and the dried seeds are the parts most traditionally used in cooking. It is a multipurpose herb grown mainly for its foliage and seeds which have numerous food-related biological activities and multiple functional uses [2]. Its green foliage, containing proteins, vitamins, minerals (like calcium, phosphorus, and iron), fibers and carbohydrates, is used as vegetable, while both the leaves and seeds contain essential oil, rich in varying components, which provides typical flavor, when added to the food products and acts as preservative [3]. Since the coriander seeds have strong and typical scent, they are appreciated worldwide as basic ingredients of many traditional foods, particularly curry powder [4]. Coriander fruits (seeds) contain 10 to 27.7% oil and up to 2.6% essential oil, which may be used in many industrial purposes [5]. The quality of coriander is mainly determined by the essential oil content and its composition. Linalool is the main volatile compound in coriander seeds, typically constituting more than 50% of the total essential oil [6]. The essential oil and various extracts from coriander fruits have anti-bacterial [7], antioxidant [8], anti-diabetic [9], anti-cancerous and anti-mutagenic activities [10]. It can also act as a sedative or for relief of nervousness.
Water stress is one of the most important environmental stresses that alter the physiological

and biochemical properties of plants and limit their growth and production. Adverse effect of water deficiton plants depends on the intensity and duration of water stress and plant growth stages [11]. Drought stress occurs in the plant when water intake is less than transpiration. This is possible due to excessive water loss, absorption reduction or both of them [12]. Plants under water stress display several morphological, physiological and molecular responses [13]. One of the physiological responses that plants use against drought is proline accumulation. Accumulation of small compatible solutes (osmolytes) such as proline helps the cells to maintain their dehydrated state and the structural integrity of the membranes to provide resistance against drought and cellular dehydration [14]. Changes in essential oils extracted from aromatic plants and their composition have been observed under water stress [15]. Salicylic acid (SA) can be utilized for the induction of plant defense system that enables the plant to withstand many biotic and abiotic stresses [16].

Salicylic acid or ortho-hydroxy benzoic acid is a natural phenolic compound and an endogenous growth regulator that affects various physiological and biochemical activities of plants [17] such as stomatal closure, photosynthesis, ion uptake, inhibition of ethylene biosynthesis and transpiration [18, 19]. The effects of SA on plant physiological processes depend on plant species and developmental phase, concentration, method and time of SA application [20]. Action mechanism of salicylic acid against stress returns to its role in regulation of antioxidant enzymes and compounds containing active oxygen species in plant [21]. Sharafizad *et al.* [22] reported that exogenous application of salicylic acid alleviated the adverse effect of drought stress in wheat plants. Significant effects of salicylic acid in increasing the tolerance of plants to drought stress have been also reported in *Plantagoovata* Forssk [23] and *Artemisia aucheri* [24]. Bio-fertilizers can also increase plant resist to environmental stresses.

Nitrogen is a major nutrient for all plants. Nitrogen accumulation in plants does not depend on whether the plants are grown in N-fertilized or non-fertilized plots [25]. This finding leads to the hypothesis that the cultivation of coriander in soils containing limited amounts of nitrogen without fertilization will likely lead to an unacceptable depletion of nitrogen. Thus, the sustainable cultivation of coriander should be performed to regenerate the nitrogen content of soils through a proper choice of crop rotation or through fertilization. In addition, some aspects of the coriander plant architecture and physiology pose some difficulties associated with the proper management of N in the field. In fact, similar to other medicinal and aromatic plants, coriander is often considered to behave as a "weedy" species. However, the increasing use of fertilizers and high productive systems have also created environmental problems such as deterioration of soil quality, surface water and groundwater, as well as air pollution, reduced biodiversity, and suppressed ecosystem function [26]. Bio-fertilizers are the newest and best way of supplying mineral nutrients for crops.

Application of bio-fertilizers leads to a decrease in the use of chemical fertilizers and provides high quality products free of harmful agrochemicals for human safety [27]. Bio-fertilizer are microbial preparations containing living cells of different microorganisms which when applied to seed, plant surface, or soil, colonizes the rhizosphere or the anterior of the plant and promotes growth by increasing the supply or availability of nutrients to the host plant [28]. They play an important role in improving the soil fertility and have the ability to mobilize nutrients in soil from unusable to usable form through biological process [29]. Besides their role in atmospheric nitrogen fixation and phosphorous solubilisation, bio-fertilizers also help in stimulating the plant growth hormones, providing better nutrient uptake and increasing plant tolerance towards drought stress [30]. Nitrokara is a bio-fertilizer that its efficiency of 1 kg or 1 liter is equal to 100 kg urea fertilizer.

Nitrokarais a non-chemical composition that has highly efficient nitrogen fixing bacteria called *Azorhizobium caulinodans* which was isolated from nature. *Azorhizobium Caulinodans s*produces growth promoting substances such as indoleacetic acid (IAA) and gibberellins, and promotes root proliferation that increases the rootlet density and root branching. This bio-fertilizer promotes the growth of plants, reduces the need for artificial chemicals in fields and gardens, improves soil structure and water holding capacity and restores natural soil fertility [31]. It has been reported that bio-fertilizers have favorable effects on growth, yield and chemical composition of medicinal plants [32, 33].

Since the effects of chemical and biological nitrogen fertilizers and salicylic acid on yield, percentage and composition of essential oil of coriander seeds under different irrigation treatments were not documented, this research was aimed to detect these effects on this important vegetable and medicinal plant.

## 2. Materials and Methods

### 2.1 Experimental design and field conditions

Two experiments as split-plot factorial based on randomized complete block design with three replications was conducted in 2014 and 2015 at the Research Farm of Kermanshah, Iran (latitude 47°34′N, longitude 34°39′E, altitude 1200 m above sea level) to evaluate the effects of bio-fertilizer and salicylic acid on yield and contents of proline and essential oil of coriander under different irrigation treatments. Average monthly temperature and rainfall during the experiment in 2014 and 2015 are shown in Table 1. The soil was loamy with field capacity of 28.4%. Soil test results of the experimental area are presented in Table 2.

**Table 1:** Average monthly temperature and rainfall during experiment in 2014 and 2015

| Month | Temperature (°C) | | | | | | Number of rainy days | | Rainfall (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | | Max | | Mean | | | | | |
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| 21 MAR - 20 APR | -6.6 | -2.6 | 27.4 | 26.2 | 10.7 | 10.5 | 6 | 10 | 22.9 | 75.9 |
| 21 APR - 21 MAY | 3.4 | -1.2 | 30.0 | 31.5 | 16.6 | 16.9 | 5 | 4 | 15.2 | 14.5 |
| 22 MAY - 21 JUN | 4.0 | 7.0 | 37.0 | 36.7 | 21.3 | 23.1 | 3 | 2 | 11.9 | 5.7 |
| 22 JUN - 22 JUL | 9.8 | 8.8 | 40.5 | 41.0 | 27.2 | 27.7 | 0 | 0 | 0.0 | 0.0 |
| 23 JUL - 22 AUG | 11.2 | 12.2 | 41.0 | 39.2 | 27.0 | 27.1 | 0 | 0 | 0.0 | 0.0 |

**Table 2:** Physical and chemical characteristics of research field soil

| Soil | Sand (%) | Clay % | Silt (%) | EC ($dSm^{-1}$) | pH | OC (%) | Fe (ppm) | K ($mgkg^{-1}$) | P ($mgkg^{-1}$) | N (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Loamy | 29 | 26 | 45 | 0.4 | 8.09 | 2 | 1.38 | 232 | 14.1 | 0.2 |

### 2.2 Treatments
Irrigation treatments ($I_1$, $I_2$ and $I_3$: irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively) were located in main plots and combination of nitrogen fertilizers (control, urea 100 $kgha^{-1}$, Nitrokara (as bio-fertilizer) and 50% urea + Nitrokara) and salicylic acid (0 and 1 mM) were allocated to sub plots.

### 2.3 Experimental conditions
Before sowing, coriander seeds were inoculated with Nitrokara; a bio-fertilizer containing *Azorhizobium caulinodans* bacteria with a formulation of Kara technique living industrial company. Each plot had 6 rows of 4 m length, spaced 20 cm apart. The seeds were sown by hand on May 2nd 2014 and April 9th 2015 with a density of 40 seeds per $m^2$. 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. Salicylic acid (0 and 1 mM) was sprayed on plants at two stages of stem elongation and flowering using a hand pump sprayer at the time of 07:00 to 08:00AM, until both sides of the leaves completely and uniformly became wet. Weeds were controlled by hand during plant growth and development as required.

### 2.4 Measurements
Free proline was determined according to Bates *et al*. [34]. At the flowering stage, 500 mg of fresh material was ground with liquid nitrogen in a mortar andhomogenized with 10 ml aqueous sulfosalicylic acid (3%w/v).The homogenates were centrifuged at 13000 rpm for 10 min. Then 2 ml of extracted solution was reacted with anequal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 ml of glacial aceticacid and 20 ml 6 M $H_3PO_4$) and incubated at 95 °C for1 h. The reaction was terminated by placing the containerin an ice bath. Subsequently, 4 ml toluene was added to the mixture and vigorously vortexed for 15 secand the samples were then kept in a room with 20-25 ºC for at least 20 min to separate the toluene and aqueous phase. Finally, the absorbance of pink-red upper phase containing prolinewas read by Spectrophotometer DR/4000 at 520 nm against a toluene blank using L-proline as a standard. The concentration of leaves proline was calculated using a standard curve.

In both years, at the physiological maturity, plants of 1 $m^2$ in the middle part of each plot were harvested and grain yield per unit area was recorded. Then, 100 g of air-dried and ground seeds from each treatment were mixed with 500 ml distilled water and the essential oil content was determined by hydro-distillationfor three hours, using a modified Clevenger apparatus and subsequently essential oil yield for every treatment at each replicate was determined.

The extracted essential oil was dried over anhydrous sodium sulphate and stored in a dark glass and kept at a temperature of 4 °C, until gas chromatography-mass spectrometry (GC/MS) analysis. In the second year, the analysis was conducted using a Thermoquest-Finnigan Trace GC/MS instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min and then kept at 250 °C for 10 min. Transfer line temperature was 250 °C. 1 μl of sample was injected and helium was used as the carrier gas at a flow rate of 1.1 ml/min with a split ratio equal to 1/50. The quadrupole mass spectrometer was scanned over the 40 to 500 *m/z*with an ionizing voltage of 70 eV and an ionization current of 150 µA.

The essential oil components were identified by comparing retention indices (RI) with authentic compounds or with those reported in the literature and by comparison of their mass spectra with the Wiley library or published mass spectra data. RI was calculated by using retention times of n-alkanes ($C_6$-$C_{24}$) that were injected after the essential oil at the same temperature and conditions. The relative percentage of the essential oil constituents was expressed by peak area normalization.

### 2.5 Statistical analysis
The data were tested for normality and homogeneity of residuals using the Kolmogorov-Smirnov and Bartlett tests, respectively. The data were analyzed by SAS software. The means were compared using the Duncan multiple range test at $p \leq 0.05$. Excel software was used to draw figures.

## 3. Results and Discussion
### 3.1 Leaf proline content
The analysis of variance of the data showed significant effects of irrigation, fertilization and foliar application of salicylic acid on leaf proline content of coriander ($p \leq 0.01$). Interactions of irrigation × fertilizer, irrigation × SAand fertilizer × SA were also significant for proline content ($p \leq 0.01$). Proline content of coriander leaves was minimal under well irrigation ($I_1$) and increased with decreasing water availability. The highest concentration of proline was recorded for plants treated with 50% urea and Nitrokara under severe water deficit ($I_3$). Leaf proline content was slightly increased with foliar application of salicylic acid under different irrigation treatments. This advantage was more evident under moderate water stress ($I_2$). Foliar application of SA also enhanced proline content of coriander leavesinuntreated plants and treated with urea, Nitrokara and 50% urea and Nitrokara up to 9.7%, 2.7%, 3% and 6.8% compared with non-application of SA in same fertilization treatments, respectively (Table. 5).

**Table 3:** Means of proline content, and essential oil percentage and yield of coriander for irrigation × fertilization

| Irrigation intervals | Fertilization treatments | Proline content (µg g$^{-1}$) | Essential oil (%) | Essential oil yield (kg ha$^{1}$) |
|---|---|---|---|---|
| I1 | C | 12.55 j | 0.26 g | 3.4 de |
| | U | 12.98 i | 0.29 f | 4.5 b |
| | N | 13.00 i | 0.29 f | 4.5 b |
| | U+N | 13.25 h | 0.30 ef | 5.2 a |
| I2 | C | 14.58 g | 0.29 f | 2.9 f |
| | U | 14.69 f | 0.31 e | 3.9 cd |
| | N | 14.79 e | 0.32 de | 4.1 c |
| | U+N | 14.85 e | 0.34 cd | 4.5 b |
| I3 | C | 15.30 d | 0.33 d | 2.3 g |
| | U | 15.59 c | 0.35 c | 3.2 e |
| | N | 15.73 b | 0.37 b | 3.5 de |
| | U+N | 16.11 a | 0.39 a | 3.7 d |

$I_1$, $I_2$, $I_3$: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively
C, U, N, U+N: Control, urea (100 kg ha$^{-1}$), Nitrokara, and 50% urea + Nitrokara, respectively
Different letters in each column indicate significant difference at $p \leq 0.05$

**Table 4:** Means of proline content, and essential oil percentage and yield of coriander for irrigation × SA

| Irrigation intervals | Salicylic acid (SA) | Proline content (µg g$^{-1}$) | Essential oil (%) | Essential oil yield (kg ha$^{-1}$) |
|---|---|---|---|---|
| I1 | Control | 12.85 f | 0.25 f | 4.0 c |
| | SA (1mM) | 12.94 e | 0.27 e | 4.7 a |
| I2 | Control | 14.77 d | 0.29 d | 3.6 d |
| | SA (1mM) | 14.89 c | 0.30 c | 4.4 b |
| I3 | Control | 15.81 b | 0.34 b | 2.8 f |
| | SA (1mM) | 15.89 a | 0.38 a | 3.2 e |

$I_1$, $I_2$, $I_3$: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively
Different letters in each column indicate significant difference at $p \leq 0.05$

**Table 5:** Means of proline content, and essential oil percentage and yield of coriander for fertilization × SA

| Fertilization treatments | Salicylic acid (SA) | Proline content (µg g$^{-1}$) | Essential oil (%) | Essential oil yield (kg ha$^{-1}$) |
|---|---|---|---|---|
| C | Control | 13.10 f | 0.27 e | 2.2 g |
| | SA (1mM) | 14.37 e | 0.30 d | 3.3 f |
| U | Control | 14.62 d | 0.31 cd | 3.8 e |
| | SA (1mM) | 15.02 b | 0.32 c | 4.8 c |
| N | Control | 14.66 d | 0.32 c | 4.3 d |
| | SA (1mM) | 15.10 b | 0.34 b | 5.6 b |
| U+N | Control | 14.78 c | 0.33 bc | 4.9 c |
| | SA (1mM) | 15.79 a | 0.36 a | 6.5 a |

C, U, N, U+N: Control, urea (100 kg ha$^{-1}$), Nitrokara, and 50% urea + Nitrokara, respectively
Different letters in each column indicate significant difference at $p \leq 0.05$

One of the physiological responses of plants to water stress is synthesis and accumulation of proline that can help maintain water absorption and cell turgor pressure. High proline content in coriander leaves under water stress (Fig. 1) may be an adaptive feature for improving its succulence and maintaining the water balance in this condition. Accumulated proline might act as a compatible solute regulating and reducing water loss from the plant cell during water deficit [35]. Increasing leaf proline content due to utilization of 50% urea fertilizer + Nitrokara (Fig. 1) could be attributed to bio-fertilizers role in atmospheric nitrogen fixation and phosphorous solubilizing, stimulating the plant growth hormones, providing better nutrient uptake and consequently increasing tolerance to drought stress [30]. The increment of proline content as a result of salicylic acid application may be related to salicylic acid role in the defense response to pathogen attack and biotic and abiotic stresses in plant species [36]. Because SA increases the abscisic acid content, leading to the accumulation of proline. Misra and Misra [37] have shown that up-regulation of proline biosynthesis enzymes (such as pyrroline-5-carboxylate reductase and γ-glutamyl kinase) and down-regulation of proline oxidase activity were responsible for increased proline level in plants treated with SA.

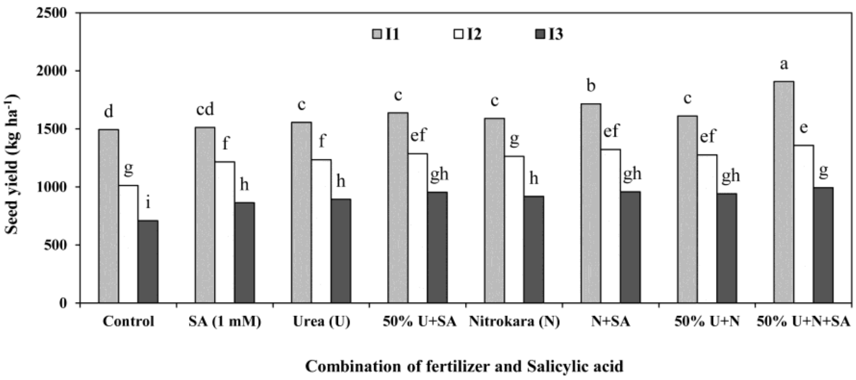
### 3.2 Seed yield

Seed yield per unit area significantly affected by year, irrigation, nitrogen fertilizers and salicylic acid application ($p \leq 0.01$). Interactions of irrigation × fertilizer, irrigation × SA, fertilizer × SA and irrigation × fertilizer × SA were also significant for this trait ($p \leq 0.01$). Seed yield of coriander plants in the second year was 18% more than that in the first year (Table 3) which can bedue to early planting and high rainfall during seedling emergence in 2015 (Table 1). The early emergence of seedlings results in more efficient and longer use of light and soil resources during the growth and development of plants. Seed yield of coriander plants decreased with increasing irrigation intervals under all fertilization and hormonal treatments. Maximum seed yield under all irrigation treatments were related to application of 50% urea +Nitrokara + SA. So that in the plants irrigated with 60 mm ($I_1$), 90 mm ($I_2$) and 120 mm evaporation intervals ($I_3$), combined application of SA, 50% urea and Nitrokara led to 27.7% 34.1% and 39.9% increase in seed yield, compared with control, under same irrigation treatments, respectively (Fig. 1).

**Table 6:** Means of yields of seed and essential oil of coriander in 2014 and 2015

| Year | Seed yield (kg ha$^{-1}$) | Seed essential oil yield (kg ha$^{-1}$) |
|---|---|---|
| 2014 | 1603 b | 5.7 b |
| 2015 | 1892 a | 6.4 a |

Different letters in each column indicate significant difference at $p \leq 0.05$




$I_1$, $I_2$, $I_3$: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively
Different letters in each column indicate significant difference at $p \leq 0.05$
W Different letters indicate significant difference at $p \leq 0.05$

**Fig 1:** Means of seed yield of coriander affected by irrigation × fertilizer × salicylic acid (SA)

Water stress reduced seed yield of coriander plants due to the reduction of leaf area, chlorophyll content and plants biomass under this conditions [38]. 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 growth and yield of plants 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 [39]. The growth promoting effects of SA could be related to changes in the hormonal status [40] or improvement of photosynthesis, transpiration and stomatal conductance [41].

### 3.3 Essential oil percentage and yield

Irrigation intervals, fertilization, SA application, and interactions of irrigation × fertilizer and irrigation × SA had a significant effect on essential oil content and yield of coriander seeds ($p \leq 0.01$). Effect of year was only significant for essential oil yield ($p \leq 0.01$).Interaction of fertilizer × SA was also significant for essential oil content ($p \leq 0.05$) and essential oil yield ($p \leq 0.01$). The essential oil content of coriander seeds under different treatments was 0.25 to 0.39%. Like seed yield, essential oil yield of coriander seeds in the second year was higher than that in the first year (Table 3). Water deficit significantly increased the essential oil percentage of coriander seeds. The highest essential oil content was related to plants treated with 50% urea and Nitrokaraunder all irrigation treatments. Increasing irrigation intervals reduced essential oil yield of all fertilized plants. This reduction was considerable for untreated plants (control plants) in comparison with plants treated with fertilizers. Plants irrigated with 120 mm evaporation intervals ($I_3$) showed better response to application of nitrogen fertilizers in terms of essential oil yield. However under well watering ($I_1$), plants had higher essential oil yield compared with other irrigation treatments (Fig.3).The percentage and yield of seed essential oil increased by foliar application of SA under all irrigation treatments. Maximum essential oil percentage and minimum essential oil yield were recorded for plants irrigated with 120 mm evaporation intervals ($I_3$). Essential oil content of plants treated with SA under well watering ($I_1$), moderate ($I_2$) and severe water deficit ($I_3$) was8%, 3.4% and 11.8%higher than that in untreated plants ($SA_0$), respectively (Fig. 4).The application of SA improved essential oil percentage and yield of coriander seeds under different fertilizations, however changes in essential oil content was lower than essential oil yield. The advantage of SA application was more evident in untreated plants, however highest essential oil percentage and yield were recorded for plants treated with SA and 50% urea + Nitrokara Fig. 5).

Accumulation of secondary metabolites is known as a defense mechanism of plants and plants can respond and adapt to the water stress by altering their cellular metabolism to invoke various defense mechanisms [42]. Although essential oil percentage of coriander seeds increased as a result of water stress, essential oil yield decreased under stressful condition (Fig.3), mainly due to considerable reduction in seed yield in this condition (Fig.2). Changes in the content of essential oils extracted from aromatic plants and their composition has been observed under water stress [43]. In chamomile, the essential oil components increased whereas the essential oil yield decreased in response to severe drought stress [44]. Improvement of essential oil and proline contents with increasing stress levels have been also reported in *Salvia officinalis* L. [45]. Increasing seed yield by application of salicylic acid and fertilizers (Fig. 2) also resulted in higher essential oil yield under all irrigation treatments. Thus, combined use of fertilizers and salicylic acid can improve coriander performance under a wide range of environmental conditions.

Salicylic acid can regulate various plant metabolic processes and modulate the production of varied osmolytes and secondary metabolites, and also maintain plant-nutrient status hence, to protect plants under abiotic stress conditions. SA can regulate many aspects in plants at gene level and thereby can improve plant-abiotic stress tolerance [46].

Exogenous application of SA has been also increased growth, yield and essential oil content of basil and marjoram [47]. The superiorities of SA treated coriander plants in seed and essential oil yields (Fig 2, 4) directly related with enhanced chlorophyll content and LAI under favorable and stressful conditions [38].

### 3.4. Main components of essential oil
Forty two different compounds, representing more than 98% of the total chemical composition of the essential oil, were identified in essential oil of different treatments. According to Table 4, Linalool was main component inessential oil of coriander seeds under all treatments.γ-terpinene, α-pinene, β-pinene and *p*-cymene were other major components in essential oil. Decreasing water availabilityled to as light increment in concentration of all major compounds of essential oil, except γ-terpinene. Under all irrigation intervals, the lowest percentage of Linalool was related to untreated plants. Application of salicylic acid and nitrogen fertilizer (especially 50% urea + Nitrokara) improved quality of coriander essential oil by increasing the percentage of Linalool and γ-terpinene. In contrast, α-pinenecontent was reduced in seed essential oil of plants treated with SA. The maximum percent of Linalool (78.04%) was related to combination application of SA, 50% urea and Nitrokara in the plants irrigated with 120 mm evaporation intervals ($I_3$). While, the highest γ-terpinene content (6.53%) was observed in plants treated with SA, 50% urea and Nitrokaraunder well watering ($I_{60}F_3SA_1$).

**Table 7:** Major components of essential oil of coriander seeds under different treatments (Means of two replications)

| Treatments | Linalool (%) | γ-terpinene (%) | α-pinene (%) | β-pinene (%) | *p*-cymene (%) |
|---|---|---|---|---|---|
| $I_{60}F_0SA_0$ | 68.81 | 5.14 | 3.33 | 0.112 | 0.56 |
| $I_{90}F_0SA_0$ | 69.53 | 4.97 | 3.65 | 0.137 | 0.63 |
| $I_{120}F_0SA_0$ | 71.00 | 4.69 | 3.77 | 0.169 | 0.71 |
| $I_{60}F_1SA_0$ | 71.16 | 5.69 | 3.83 | 0.151 | 0.65 |
| $I_{90}F_1SA_0$ | 73.22 | 5.21 | 3.98 | 0.179 | 0.74 |
| $I_{120}F_1SA_0$ | 74.51 | 5.05 | 4.01 | 0.197 | 0.80 |
| $I_{60}F_2SA_0$ | 73.28 | 5.74 | 3.90 | 0.168 | 0.69 |
| $I_{90}F_2SA_0$ | 74.55 | 5.63 | 4.05 | 0.226 | 0.78 |
| $I_{120}F_2SA_0$ | 75.19 | 5.50 | 4.15 | 0.241 | 0.85 |
| $I_{60}F_3SA_0$ | 74.90 | 6.15 | 4.12 | 0.244 | 0.81 |
| $I_{90}F_3SA_0$ | 75.15 | 5.93 | 4.20 | 0.292 | 0.97 |
| $I_{120}F_3SA_0$ | 76.27 | 5.83 | 4.55 | 0.339 | 0.94 |
| $I_{60}F_0SA_1$ | 71.09 | 5.37 | 3.15 | 0.143 | 0.62 |
| $I_{90}F_0SA_1$ | 72.94 | 5.12 | 3.30 | 0.174 | 0.71 |
| $I_{120}F_0SA_1$ | 73.67 | 4.95 | 3.41 | 0.201 | 0.79 |
| $I_{60}F_1SA_1$ | 75.21 | 5.64 | 3.42 | 0.185 | 0.72 |
| $I_{90}F_1SA_1$ | 76.40 | 5.47 | 3.57 | 0.215 | 0.81 |
| $I_{120}F_1SA_1$ | 76.93 | 5.22 | 3.63 | 0.237 | 0.87 |
| $I_{60}F_2SA_1$ | 77.53 | 6.19 | 3.50 | 0.205 | 0.73 |
| $I_{90}F_2SA_1$ | 76.33 | 5.88 | 3.65 | 0.266 | 0.81 |
| $I_{120}F_2SA_1$ | 77.11 | 5.62 | 3.70 | 0.281 | 0.95 |
| $I_{60}F_3SA_1$ | 76.00 | 6.53 | 3.69 | 0.280 | 0.90 |
| $I_{90}F_3SA_1$ | 76.57 | 6.18 | 3.81 | 0.338 | 1.02 |
| $I_{120}F_3SA_1$ | 78.04 | 6.04 | 3.95 | 0.382 | 1.31 |

$I_1$, $I_2$, $I_3$: Irrigation after 60, 90 and 120 mm evaporation from class A pan, respectively $F_0$, $F_1$, $F_2$, $F_3$: No fertilizer (control), urea, Nitrokara (bio-fertilizer), and 50% urea +Nitrokara, respectively $SA_0$ and $SA_1$: Non-application of salicylic acid and application of 1mM salicylic acid, respectively

Secondary metabolites play a major role in the adaptation of plants to the environment changes [47]. Their biosynthesis is not only controlled genetically, but is also strongly affected by environmental parameters [48]. Aromatic plants represent a renewable source of flavoring substances, which may be used in the food, perfumery and pharmaceutical industries [49]. The volatile oil of coriander is utilized in different branches of the food industry such as bakery, meat processing, and chewing gum production. This essence is also extensively used in perfumery and in the cosmetic industry for its powerful aroma [50] and in the pharmaceutical industry [51]. Linalool, a terpene tertiary alcohol, has antioxidant potency at high concentrations [52]. Linalool content in the coriander seed essential oil has been reported very different. It was 40% [53], 55% [54], 57-59% [55], 73.8% [56], 70-75% [57] and 72-83% [58] in different researches.

The high concentration of Linalool in seed essential oil makes it respectively potentially useful in the medicines and perfumery purposes. In addition to its use as a scent in domestic products such as soap, detergent, shampoo, and lotion, linalool is also used as a chemical intermediate. One common downstream product of linalool is vitamin E. Linalool is also used by pest professionals as a flea, and cockroach insecticide [59]. Therefore, it may be concluded that coriander growing widely in Iran, may be utilized as a source for the isolation of natural Linalool.

SA is involved in various physiological processes in plants, such as growth regulation, photosynthesis, stomatal conductance, nutrient uptake, plant water relations and mechanisms of plant resistance and tolerance to biotic and abiotic stresses. It affects ethylene biosynthesis, stomatal movement and also reverses the effects of abscisic acid on leaf abscission, enhancement of the level of chlorophyll and carotenoid pigments. Enhancement of photosynthetic rate and modifying the activity of some of the important enzymes, are the other roles assigned to SA. Salicylic acid may change secondary metabolites through its effect on plastid, chlorophyll level and also simulating stress conditions [60]. Exogenous SA were effective in inducing secondary metabolites formation in plant cell culture or in vivo plants [61].

Increment of main compositions in essential oil of coriander seeds (such as linalool, γ-terpinne andα-pinene) has been reported in the presence of bio-fertilizers [62].

**4. Conclusions**

Water deficit caused a significant reduction in seed and essential oil yields and a significant increment in leaf proline and essential oil content of coriander seeds. On the other hand, application of salicylic acid and nitrogen fertilizers, especially 50% urea + Nitrokara, had an additive effect on seed and essential oil yields under all irrigation treatments. Forty two different compounds were identified in essential oil of different treatments. The main constituent of coriander essential oil under all treatments was Linalool. Decreasing water availability led to a slight increment in concentration of all major compounds of essential oil, except γ-terpinene. Application of SA and nitrogen fertilizer (especially 50% urea + Nitrokara) improved percent and quality of coriander essential oil by increasing the percentage of linalool and γ-terpinene. Since nitrogen bio-fertilizers can supplement only a part of the nitrogen requirement of the inoculated plants, low dose of nitrogen as the recommendation may be applied. Therefore, salicylic acid treatment alleviates the negative effects of water stress and can be recommended for improving field performance of coriander under different water availabilities when applied with bio-fertilizers.

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