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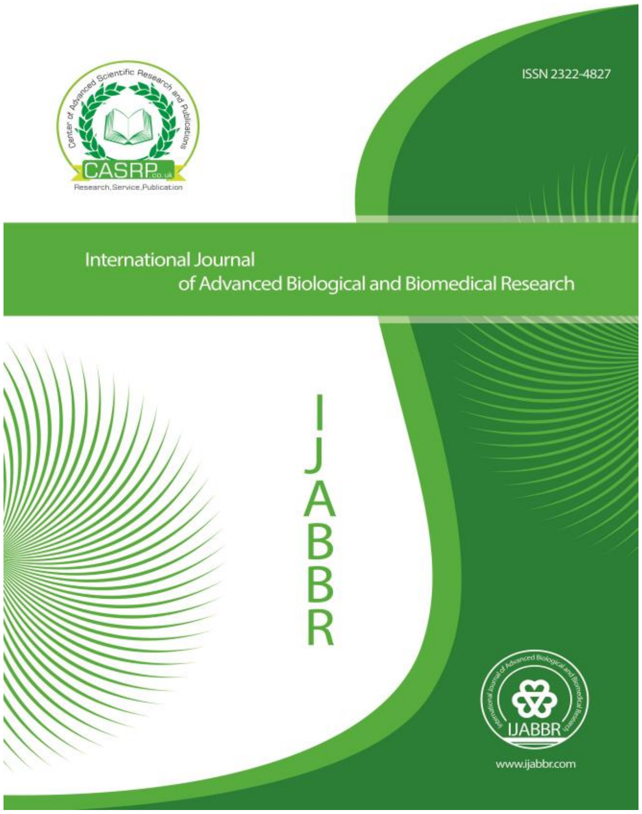

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Changes in growth, chlorophyll content and grain yield of Coriander (*Coriandrum sativum* L.) in response to water stress, chemical and biological fertilizers and salicylic acid

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Abstract

Salicylic acid (SA) is a phyto-hormone that regulates physiological and biological processes in plants and can be used to improve plant growth under different environmental conditions, including water stress. Thus, a field experiment as split plot factorial based on randomized complete blocks design with three replications was conducted in 2014 to investigate the effects of fertilizer and salicylic acid on growth, chlorophyll content and grain yield of coriander (*Coriandrum sativum* L.) plants under drought stress. Treatments were three levels of water supply (irrigation after 60, 90 and 120 mm evaporation from class A pan) and four levels of fertilizer application (control, 100 kg ha^{-1} Urea, Nitrokara (biofertilizer) and 50% Urea + Nitrokara) and foliar spray of salicylic acid (0 and 1 mM). Results indicated that water deficit had a significant reduction effect on leaf area, chlorophyll content, fresh weight of root and leaf and grain yield. Salicylic acid and 50% Urea + Nitrokara resulted in a significant increase of all traits under stress and well watering conditions. Although highest amount of studied traits was observed in plants treated with SA or application of 50% Urea and Nitrokara under well watering, but the changes rate was higher under stress conditions. Therefore, salicylic acid and combination of urea and Nitrokara can be used to promote growth of coriander under different water availabilities, which ultimately can enhance field performance of this plant.

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Keywords: Fertilizer, Chlorophyll, Coriander, Salicylic acid, Water stress, Yield.

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1. Introduction

Water stress is the most factor restricting plants growth and production in the world. The plants response to water shortage is very complex, which is either adoptive or detrimental and it depends on stress degree (intensity and duration), plant species and stage of their development (Demirevska et al., 2009). Plants under drought stress conditions struggle to revise their metabolic and structural capabilities mediated by modified gene expression which assists to improve their potential under stress environment (Bohnert and Sheveleva, 1998). Water deficit reduces turgor pressure in plant cells, their division and elongation, thereby strongly limits plant growth (Baigorri et al., 1999). Leaf characteristics play critical role in determining rates of photosynthesis and transpiration (Hopkins et al., 2008). Increasing of water stress reduces leaf area that leads to less light absorption, leaf chlorophyll content, photosynthesis, growth and yield.

Coriander (*Coriandrum sativum*) is an annual herb from Apiacea family, which possesses nutritional and medicinal properties. It is one of the most commonly used spices (Leena et al., 2012), that is known as a culinary and medicinal plant native to the Mediterranean and Middle Eastern regions. It is a multipurpose herb grown mainly for its foliage and grains, which have numerous food-related biological activities and multiple functional uses (Burdock and Carabin, 2009). Coriander grains contain 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 grains 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).

Plant growth regulators such as salicylic acid, cytokinin, gibberrellins and abscisic acid amend the plant responses to water stress (Farooq et al., 2009). Salicylic acid (SA) or ortho-hydroxy benzoic acid is a phenolic phyto-hormone that its exogenous application may directly or indirectly affect various physiological and biochemical processes in plants, including stomatal regulation, photosynthesis, chlorophyll content, ion uptake (Ananieva et al., 2002), induction of antioxidant synthesis (Yordanova and Popova, 2007) and plant growth and yield (Khodary, 2004). The effect of salicylic acid on plant physiological processes depends on plant species, its developmental stage, SA concentration as well as environmental conditions (El-Shraiy and Hegazi, 2009). Salicylic acid plays an important role in the enhancement of plant resistance against different biotic and abiotic stress through various mechanisms (Horvath et al., 2007; Hayat et al., 2010; Kadioglu et al., 2011).

The application of appropriate and adequate fertilizers may significantly increase plants growth and performance in terms of quantity and quality (Sakakibara et al., 2006). A meta-analysis of the data collected from experiments performed worldwide revealed that N fertilization enhanced yield in the range of 10 to 70% regardless of the experimental variables, and that every additional 10 kg ha^{-1} N fertilizer supply generally generates an increase in seed yield from approximately 20 to 70 kg ha^{-1} (Carrubba, 2009). Although organic management is often a recommended choice for the growth of medicinal and aromatic plants (Malik et al., 2011; Naguib, 2011), few efforts have been devoted to the study of fertilization under organic crop management. Biofertilizers play a major role in the productivity and sustainability of soil and maintaining its fertility and can be a suitable alternative for chemical fertilizer to improve plant production in sustainable farming (Wu et al., 2005).

Biofertilizers are the natural product carrying living microorganisms derived from the root or cultivated soil. They are fertilizing compounds that are composed of one or more species of useful soil-living organisms and are presented on preservative substances. Biofertilizers are microorganisms that are able to change nutritional elements from useless form to effective and useful compounds and this change is conducted in a biological process. Production expenses of bio-fertilizers are low and it does not cause pollution in the soil and environment (Rahimi-Shokooh et al., 2013). Since the combined effects of salicylic acid, chemical and biological nitrogen fertilizers under water stress on coriander plant were not documented, this research was aimed to determine these effects on growth, chlorophyll content and grain yield of this plant.

2. Materials and methods

2.1. Field conditions

A split plot factorial experiment based on randomized complete block design with three replications was conducted at the Research Farm of Kermanshah, Iran (latitude 47°34′N, longitude 34°39′E, altitude 1200 m above sea level) in 2014. 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 type	Sand (%)	Clay (%)	Silt (%)	EC ($ds.m^{-1}$)	pH	OC (%)	Fe (ppm)	K ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)	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 fertilizers (control, Urea 100 $kg.ha^{-1}$, Nitrokara (biofertilizer) and 50% Urea + Nitrokara) and salicylic acid (0 and 1 mM) were allocated to sub plots.

2.3. Experimental conditions and studied traits

Coriander seeds were inoculated with Nitrokara, a biofertilizer containing *Azorhizobium caulinad* bacteria with a formulation of Kara technique living industrial company, and sown by hand on April 9th 2014 with a density of 40 seeds per m^2. Each plot had 6 rows of 4 m length, spaced 20 cm apart. The distance between seed within rows was 10 cm. Urea was applied on the basis of soil test at three stages (1/3 at sowing date, 1/3 after thinning and 1/3 at vegetative stage) and salicylic acid (0 and 1 mM) sprayed on plants at two stages of stem elongation and flowering until both sides of the leaves were completely wet. All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Weeds were controlled by hand during plant growth and development as required.

At the flowering stage, 20 plants were randomly harvested from the central rows of each plot to determine coriander leaf area (LA) using a leaf area meter device. Also, 10 plants were selected from each plot and immediately transferred to the laboratory and fresh weight of leaves was recorded. Leaf chlorophyll content was also recorded using a chlorophyll meter (SPAD) at flowering stage. Measurements were done on five plants in each plot and chlorophyll content of the upper, middle and lower leaves of each plant was measured. Subsequently, the mean chlorophyll content for each treatment and replicate was calculated.

At physiological maturity, plants of 1 m^2 in the middle part of each plot were harvested and grain yield per unit area was recorded. Finally, to calculate the fresh weight of root, 10 plants were randomly selected and roots were washed with distilled water and separated from the aerial organ. Then, water on the root surface was quickly and carefully dehumidified and root fresh weight was determined with a digital scale. The data were analyzed by SPSS 16 software and the means were compared using the Duncan's multiple range test at $p \leq 0.05$. The relationships between grain yield and other traits were determined using simple correlation coefficient analysis on the average data. Excel software was used to draw the figures.

3. Results and discussion

3.1. Leaf area (LA)

Leaf area was significantly affected by irrigation, fertilizer and SA application. The interactions of irrigation × fertilizer, irrigation × SA and fertilizer × SA were also statistically significant for LA (Table 2). Water deficit significantly reduced the leaf area of coriander. However, application of nitrogen fertilizers, especially 50% Urea and Nitrokara improve leaf area under all irrigation treatments. So that in the plants irrigated with 60 mm (I_1), 90 mm (I_2) and 120 mm evaporation intervals (I_3), application of 50% Urea and Nitrokara led to 28.8%, 34.1% and 44.1% increase in leaf area, compared to no-fertilization (F_0) under same irrigation treatments, respectively (Table 3). Similar improvement in LA of coriander was observed by foliar application of SA (Table 4). Leaf area was also increased with application of SA under different fertilizations. The highest and the lowest leaf area were recorded for plants treated with SA and 50% Urea + Nitrokara (F_3SA_1) and control (F_0SA_0), respectively (Table 5).

A reduction in leaf turgor and photosynthesis under water stress condition suppresses cell expansion and growth, leading to the diminution of leaf area (Anjum et al., 2011). The decrease in leaf area under drought stress could be considered as an avoidance mechanism which minimizes water losses (Rodriguez et al., 2005) to avoid the excessive transpiration with low stomatal density (Parsons, 1982). Smaller leaves under drought stress, decrease light absorption capacity and photosynthesis rate and eventually lead to reduction of plants growth and yield (Hsiao, 1973; Ozturk et al., 2004). Salicylic acid application promotes cell division and enlargement (Hayat et al., 2005). Delavari et al. (2010) reported that SA increases the leaf area in sweet basil plants, which is agreement of our results. Additive effect of SA on leaf area under water stress was also reported in maize (Zamaninejad et al., 2013).

3.2. Chlorophyll content

Analysis of variance showed significant effects of irrigation, fertilizer and SA application on leaf chlorophyll content. The interactions of irrigation × fertilizer, irrigation × SA and fertilizer × SA were also significant for this trait (Table 2). Chlorophyll content of coriander leaves decreased with increasing irrigation intervals, but it's improved by application of fertilizers, especially 50% Urea + Nitrokara, under all water treatments. This increment was more evident for plants irrigated with 120 mm evaporation intervals (I_3) than for plants irrigated with lower intervals (Table 3). In the all irrigation treatments, the highest chlorophyll content values were achieved in SA application. Maximum chlorophyll content was recorded for plants treated with SA under well watering. However, the effect of SA application in the improvement of chlorophyll content of coriander leaves was higher under severe water stress conditions (Table 4). Foliar application of SA had a positive and additive effect on chlorophyll content under all fertilization treatments. Salicylic acid increased chlorophyll content of untreated plants (F_0) and plants treated with Urea (F_1), Nitrokara (F_2) and 50% Urea + Nitrokara (F_3) by 13.1%, 21.5%, 21.8% and 34.3%, compared to control (F_0SA_0), respectively (Table 5).

Chlorophyll is one of the major chloroplast components for photosynthesis and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Anjum et al., 2011). The reduction in chlorophyll concentration is identified as a drought response mechanism to minimize light absorption by chloroplasts (Pastenes et al., 2005). It can be also attributed to the sensitivity of this pigment to increasing environmental stresses, especially drought and salinity (Guerfel et al., 2009), which has been reported by other researchers (Pandey et al., 2003; Sayyari et al., 2013).

It was stated that reduction of chlorophyll content under stress conditions was because of changes in nitrogen metabolism due to synthesis of compounds such as proline, which has a role in osmotic adjustment (Afshari et al., 2013). Agarwal et al. (2005) demonstrated the enhanced chlorophyll levels when the wheat leaves were treated with SA under water stress. Application of SA significantly increased growth parameters, photosynthetic pigments and proline content and decreased lipid peroxidation in sweet basil under stress conditions (Delavari et al., 2010). Nitrogen is the most important component of the chlorophyll. Therefore, low rate of photosynthesis under conditions of nitrogen limitation can be attributed to the reduction of chlorophyll content (Toth et al., 2002). For this reason, application of nitrogen (as biofertilizer or chemical), increased the chlorophyll content in coriander leaves.

3.3. Fresh weight of root and leaf

Irrigation intervals, fertilizer and SA application had significant effects on fresh weight of root and leaf. The interactions of irrigation × fertilizer, irrigation × SA and fertilizer × SA were also significant for these traits (Table 2). Fresh weight of root and leaf reduced with reduction in water availability, but application of different nitrogen fertilizer, especially 50% Urea and Nitrokara, improved fresh weight of root and leaf of coriander. This improvement for plants irrigated with 120 mm evaporation intervals (I_3) was greater than that under other irrigation treatments (Table 3). Foliar application of SA under all irrigation treatments increased fresh weight of root and leaf. The highest root fresh weight (0.49 g) and leaf fresh weight (1.810 g) were observed by application of SA under well watering. The lowest fresh weigh of root (0.23 g) and leaf (1.094 g) were related to non-salicylic acid use in plants irrigated with 120 mm evaporation intervals (I_3) (Table 4). Fresh weight of root and leaf were also enhanced with application of SA under different fertilizations. The plants treated with SA and 50% Urea + Nitrokara had highest fresh weight of root and leaf. Salicylic acid application increased root fresh weight of untreated plants (F_0) and plants treated with Urea (F_1), Nitrokara (F_2) and 50% Urea + Nitrokara (F_3) by 19.2%, 73.1%, 76.9% and 96.1%, compared to control (F_0SA_0), respectively, while changes in the fresh weight of leaf with application of SA was less than root in similar conditions (12.1, 16.9, 20.2 and 28.2%, respectively).

Cell division, enlargement and differentiation are the main processes that determine the quality and quantity of plant growth, affected by various internal and external factors, such as water stress (Patel and Golakia, 1988). The reduction in fresh weight of coriander under water stress condition may be attributed to suppression of cell expansion and cell growth due to the low turgor pressure (Sayyari et al., 2013), less expansion of leaf area, lower chlorophyll content (Table 2,3), decrease in the values of net assimilation rate (Rodriguez et al., 2005) and also more leaf senescence in this condition (Sayyari et al., 2013).

The result of this research is in agreement with the findings of Khodary (2004) and Boroumand-Jazi et al. (2011) who reported that application of SA increased the fresh and dry weight of shoot and roots under stress conditions. Ion uptake and transport (Harper and Balke, 1981), photosynthesis rate, stomatal conductivity and transpiration (Khan et al., 2003) could be affected by SA application.

Table 2
Analysis of variance of the effects of irrigation, fertilizer and salicylic acid on growth and grain yield of coriander.

		Mean Squares				
S.O.V	**df**	**Leaf area**	**Chlorophyll content**	**Root fresh weight**	**Leaf fresh weight**	**Grain yield**
Replication	2	0.842^{ns}	6.30^{ns}	0.00165^{ns}	0.0332^{ns}	8760.1^{ns}
Irrigation (I)	2	105.12^{**}	25.99^{**}	7.256^{**}	12.716^{**}	13908.3^{**}
E_a	4	0.245	1.048	0.0007	0.0155	104.1
Fertilizer (F)	3	95.81^{**}	18.32^{**}	5.050^{**}	10.134^{**}	13218.5^{**}
I×F	6	75.60^{**}	14.81^{**}	4.120^{**}	8.0116^{**}	12952.2^{**}
Salicylic acid (SA)	1	80.34^{**}	15.01^{*}	0.0040^{ns}	8.0491^{**}	13098.6^{**}
I×SA	2	70.33^{**}	12.57^{**}	4.033^{**}	7.0249^{**}	12715.7^{**}
F×SA	3	62.51^{**}	10.10^{**}	3.050^{**}	5.0562^{**}	12549.4^{**}
I×F×SA	6	8.41^{ns}	0.063^{ns}	0.0011^{ns}	0.0979^{ns}	11249.3^{**}
E_b	42	0.118	0.013	0.00002	0.00077	19.8
CV (%)	--	12.3	11.5	10.35	8.31	10.1

^{ns}, * and ** non-significant and significant at the 5% and 1% probability levels, respectively.

Table 3
Interaction effect of irrigation × fertilizer on leaf area, chlorophyll content and fresh weight of root and leaf of coriander.

Treatment	**Leaf area (m^2)**	**Chlorophyll content**	**Root fresh weight (g)**	**Leaf fresh weight (g)**
$I_{60}\times F_0$	5.2^{c}	27.15^{c}	0.43^{d}	1.60^{c}
$I_{60}\times F_1$	6.2^{b}	28.78^{b}	0.46^{c}	1.74^{b}
$I_{60}\times F_2$	6.1^{b}	28.90^{b}	0.49^{b}	1.76^{b}
$I_{60}\times F_3$	6.7^{a}	31.02^{a}	0.52^{a}	1.85^{a}
$I_{90}\times F_0$	4.4^{e}	23.93^{e}	0.33^{g}	1.26^{e}
$I_{90}\times F_1$	5.4^{c}	24.14^{e}	0.34^{g}	1.34^{d}
$I_{90}\times F_2$	5.3^{c}	24.38^{e}	0.38^{f}	1.39^{d}
$I_{90}\times F_3$	5.9^{b}	26.22^{d}	0.41^{e}	1.52^{c}
$I_{120}\times F_0$	3.4^{f}	18.07^{h}	0.22^{k}	1.01^{g}
$I_{120}\times F_1$	4.4^{e}	20.34^{g}	0.25^{j}	1.16^{f}
$I_{120}\times F_2$	4.3^{e}	20.52^{g}	0.28^{i}	1.19^{f}
$I_{120}\times F_3$	4.9^{d}	24.47^{f}	0.31^{h}	1.23^{e}

Different letters in each column indicate a significant difference at $p\leq0.05$ (Duncan´s Multiple Range Test). F_0: Control, F_1: Urea, F_2: Nitrokara, F_3: 50% Urea+Nitrokara. I_{60}, I_{90}, I_{120}: Irrigation after 60, 90 and 120 mm evaporation from pan class A, respectively.

Table 4

Interaction effect of irrigation × salicylic acid on leaf area, chlorophyll content and fresh weight of root and leaf of coriander.

Treatment	Leaf area (m^2)	Chlorophyll content	Root fresh weight (g)	Leaf fresh weight (g)
I_{60}× SA_0	5.1 ^{c}	25.10 ^{b}	0.41 ^{b}	1.682 ^{b}
I_{60}× SA_1	6.5 ^{a}	27.33 ^{a}	0.49 ^{a}	1.810 ^{a}
I_{90}× SA_0	4.5 ^{d}	21.51 ^{d}	0.30 ^{d}	1.350 ^{c}
I_{90}× SA_1	5.7 ^{b}	23.43 ^{c}	0.36 ^{c}	1.391 ^{c}
I_{120}× SA_0	3.3 ^{e}	19.89 ^{f}	0.23 ^{f}	1.094 ^{e}
I_{120}× SA_1	4.7 ^{d}	22.71 ^{e}	0.27 ^{e}	1.237 ^{d}

Different letters in each column indicate a significant difference at p≤0.05 (Duncan´s Multiple Range Test). SA_0: No salicylic acid, SA_1: Application of 1mM salicylic acid, I_{60}, I_{90}, I_{120}: Irrigation after 60, 90 and 120 mm evaporation from pan class A, respectively.

Table 5

Interaction effect of fertilization × salicylic acid on leaf area, chlorophyll content and fresh weight of root and leaf of coriander.

Treatment	Leaf area (m^2)	Chlorophyll content	Root fresh weight (g)	Leaf fresh weight (g)
F_0× SA_0	3.4 ^{g}	20.02 ^{d}	0.26 ^{f}	1.24 ^{d}
F_0× SA_1	4.1 ^{f}	22.65 ^{c}	0.31 ^{e}	1.39 ^{c}
F_1× SA_0	4.5 ^{e}	22.50 ^{c}	0.36 ^{d}	1.50 ^{b}
F_1× SA_1	5.4 ^{c}	24.32 ^{b}	0.45 ^{b}	1.45 ^{b}
F_2× SA_0	4.6 ^{e}	22.64 ^{c}	0.41 ^{c}	1.38 ^{c}
F_2× SA_1	5.9 ^{b}	24.39 ^{b}	0.46 ^{b}	1.49 ^{b}
F_3× SA_0	5.0 ^{d}	24.05 ^{b}	0.47 ^{b}	1.48 ^{b}
F_3× SA_1	6.3 ^{a}	26.88 ^{a}	0.51 ^{a}	1.59 ^{a}

Different letters in each column indicate a significant difference at p≤0.05 (Duncan´s Multiple Range Test). F_0: Control, F_1: Urea, F_2: Nitrokara, F_3: 50% Urea+Nitrokara, SA_0: No salicylic acid, SA_1: Application of 1mM salicylic acid.

3.4. Grain yield

Results of analysis of variance showed that Grain yield per unit area was significantly influenced by irrigation, fertilizer, SA application and interactions of irrigation × fertilizer, irrigation × SA, fertilizer × SA and irrigation × fertilizer × SA (Table 2). Grain yield of coriander plants decreased with increasing water stress under all fertilization and hormonal treatments. The plants treated with 50% Urea + Nitrokara + SA produced highest grain yield under all irrigations intervals. The highest increment in grain yield was obtained for plants treated with SA and Urea + Nitrokara under severe water stress condition. However, the highest grain yield of coriander was related to application of SA and Urea + Nitrokara under well watering (Fig. 1).

Water stress considerably decreased grain yield of coriander, due to reductions in leaf area, chlorophyll content and biomass (Tables 2 and 3). Beneficial effects of SA application on grain yield of coriander (Figure 1) may be related to leaf area expansion (Table 2), enhancing essential nutrients uptake, improving photosynthetic pigments (Table 2) and consequently enhancing photosynthesis and growth (Table 2; Ullah et al., 2012). Water stress reduces plant growth and development, leading to hampered flower production and grain filling and thus smaller and fewer grains. A reduction in grain filling occurs due to a reduction in the assimilate partitioning and activities of sucrose and starch synthesis enzymes (Anjum et al., 2011).

The profitable effects of biofertilizers 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 (Okon and Itzigsohn, 1995). The increment of growth and yield by seed inoculation with biofertilizer and application of chemical fertilizers has been also reported in anise (Gomaa and Abou-Aly, 2001).

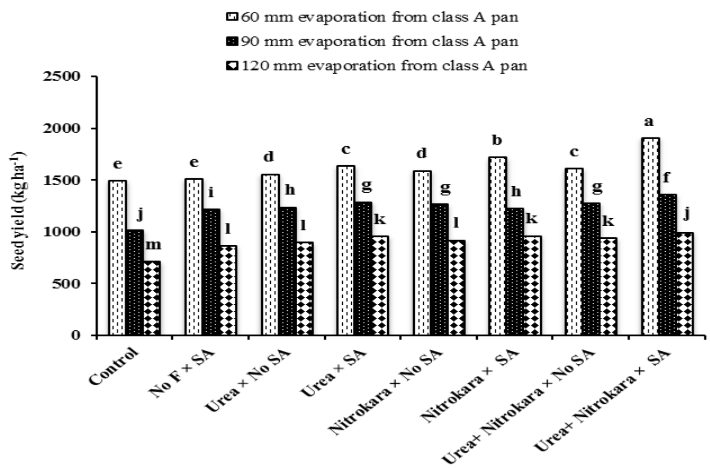

Fig. 1. Mean seed yield of coriander affected by Irrigation× fertilizer × SA Different letters indicate significant difference at p≤0.05.

3.5. Correlation between traits and grain yield

On the basis of correlation analysis results (Table 6), grain yield of coriander had a significant positive relation with leaf area (r = 0.68), chlorophyll content (r = 0.58) and leaf fresh weight (r = 0.51). Therefore, increase in these traits can simultaneously enhance grain yield of coriander. In the other words, plants with large and heavy leaves and grate chlorophyll content can produce higher grain yield. The correlation between leaf area and chlorophyll content (r = 0.55), leaf area and leaf fresh weight (r = 0.65) and chlorophyll content and leaf fresh weight (r = 0.44) were also positive and significant (Table 6).

Table 6
The correlation between studied traits and grain yield of coriander.

Traits	(1)	(2)	(3)	(4)	(6)
Leaf area (1)	1				
Chlorophyll content (2)	0.55 **	1			
Root fresh weight (3)	0.18 ^{ns}	0.10 ^{ns}	1		
Leaf fresh weight (4)	0.65 **	0.44 **	0.09 ^{ns}	1	
Seed yield (5)	0.68 **	0.58 **	0.09 ^{ns}	0.51**	1

^{ns}, * and ** : non-significant and significant at the 5% and 1% probability level, respectively.

4. Conclusion

Water stress significantly reduced leaf area, chlorophyll content, fresh weight of root and leaf and consequently grain yield per unit area of coriander decreased with increasing irrigation intervals. However, application of salicylic acid association with 50% Urea and Nitrokara increased all studied traits under stress and well watering conditions. Therefore, salicylic acid and combination of urea and Nitrokara can be used to promote growth of coriander under different water availabilities, which ultimately improve field performance.

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