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The Effects of Foliar Application of Salicylic Acid and Ascorbic Acid on Morpho-Physiological Traits of Pistachio Seedlings under Drought Stress

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A R T I C L E I N F O

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A B S T R A C T

This study was carried out to investigate the effects of foliar spray of salicylic acid and ascorbic acid on some morpho-physiological traits of *Pistacia vera* cv. Badami-zarand under drought stress. The experiment was conducted during 2017-2018 in the greenhouse condition, in a factorial randomized complete block design with three replications on pot plants. The soil texture of experimental pots was sandy-loam. Moreover, the three factors were considered as follows: first factor, drought stress treatments at three levels; control (100% usable moisture content), moderate stress (60% usable moisture content), and severe stress (30% usable moisture content); second factor, salicylic acid, at three levels (0, 0.5mM, and 1mM); and ascorbic acid at three levels (0, 0.5mM, and 1mM). At the end of experiment related physiological, morphological, and biochemical traits related to 6-month-old seedlings were assessed including the fresh and dry weight of leaf, stem and root, number of leaves, leaf thickness, diameter and length of stem, length of root, relative water content, rate of electrolyte leakage, rate of proline accumulation, rate of glycine betaine accumulation, chlorophyll a content, chlorophyll b content, and total chlorophyll. Generally, the results indicated that the value of morphological traits showed a significant reduction in stress treatments. In this experiment, it was found that the application of salicylic acid and ascorbic acid can also reduce the adverse effects of moderate drought stress. Furthermore, it was identified that the response of plant to salicylic acid treatment is similar to ascorbic acid. In general, the application of salicylic acid with a concentration of 0.5mM and ascorbic acid with concentrations of 0.5mM and 1mM is recommended to improve the adaptation of pistachio seedlings under moderate stress.

Introduction

Drought stress is one of the major problems limiting plant growth and performance and occurs when the rate of transpiration is more than the rate of water absorption (Abbaspour and Babaee, 2017; Wu and Zou, 2009; Kamanga *et al.*, 2018). In this case, all physiological and biochemical processes of plants are affected which results in plant weakness, reduced performance, reduced product quality, and eventually, death of the plant

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(Fahad *et al.*, 2017; Lotfi et al., 2019). Besides, morphological properties of plants was also shown to be affected by drought stress (Zokaee-Khosroshahi et al., 2014). The response to the water shortage depends on the species and variety of the plant, length and duration of the stress, growth and development stage of the plant (Tardieu, 2013). Plants respond to drought stress through morphological, physiological, and biochemical changes (Vahdati et al., 2009). Pistachio is a kind of tree which has been cultivating in different regions of Iran for a long time and has a special position among the agricultural products in terms of economic value as an important crop (Panahi *et al.*, 2001). Salicylic acid is a hormonal substance which plays an important role in regulating plant growth, seed germination, fruit yield, and flowering of the plants (Hajam *et al.*, 2017). Furthermore, it also plays a role in the absorption and transmission of ions (Harper and Balke, 1981), the rate of photosynthesis, porous conductance, and transpiration (Khan *et al.*, 2003). The effect of salicylic acid on defense mechanisms under biological and non-biological stresses shows that it can also be effective against drought stress by regulation of important plant physiological processes such as photosynthesis, nitrogen metabolism, proline metabolism, production of glycine betaine and plant-water relations (Khan *et al.*, 2015). Exogenously sourced salicylic acid to stressed plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying was reported to induce major abiotic stress tolerance-mechanisms (Khan et al., 2015; Shahmoradi and Naderi, 2018; Shamshiri and Hassani, 2015). Foliar application of Salicylic acid strengthened antioxidant defense system in drought-tolerant *Zea mays* cultivar to a great extent (Saruhan et al., 2012; Abbaspour and Babaee, 2017). On the other hand, ascorbic acid is a small water-soluble antioxidant which plays a role in detoxification of active oxygen species, especially hydrogen peroxide (Babashpour-Asl and Piryaei, 2021). Moreover, it directly plays a role in neutralizing the radicals of superoxide or individual oxygen and as a secondary antioxidant in the production of alpha-tocopherol and other lipophilic antioxidants (Sharma *et al.*, 2012). The foliar application of ascorbic acid could increase non-biological stress resistance and reduce the effect of oxidative stress (Sharma *et al.*, 2012). Ascorbic acid along with glutathione and some antioxidant enzymes contribute to the neutralization of active oxygen radicals, including superoxide ion resulted from various non-biological stresses (Noctor and Foyer, 1998; Seminario *et al.*, 2017). Therefore, the purpose of this experiment was to investigate the effects of salicylic acid and ascorbic acid on physiological responses of pistachio seedlings “Badami-zarand” as rootstock under drought stress.

Materials and Methods

The experiment was conducted in a factorial randomized complete block design in greenhouse condition with three replications. So three factors were considered as follows: first factor, drought stress treatments at three levels of control (100% usable moisture content), moderate stress (60% usable moisture content), and severe stress (30% usable moisture content); second factor, salicylic acid, at three levels of 0, 0.5mM, and 1mM; and ascorbic acid at three levels of 0, 0.5mM, and 1mM. The water regimes were applied based on the pot weight, soil dry weight and soil wet weight after watering and ceasing the runoff (Bagheri *et al.*, 2012). The seedlings were treated with the foliar application of various concentrations of salicylic acid and ascorbic acid. Furthermore, the soil texture used for the experimental pots was sandy-loam. At the end of experiment, 6-month-old seedlings were harvested and the physiological, morphological, and biochemical traits were assessed including the fresh and dry weight of leaf, stem and root, number of leaves, leaf thickness, diameter of stem, length of stem, length of root, relative water content (RWC), electrolyte leakage, rate of proline accumulation, rate of glycine betaine accumulation, contents of chlorophyll a, b and total chlorophyll. Fresh weight of Leaf, stem and root samples were determined by using an *Oertling* balance (UK). Then, samples were dried for 24 hours at 70°C in a *Gallenkamp* oven (UK). The dry weights of samples were determined by using an *Oertling* balance (UK). The leaf thickness was measured according to Hajiboland *et al.* (2014) method. The diameter of stem, length of stem and length of root was measured according to Hajiboland *et al.* (2014) method. The relative water contents (RWC) of leaves were determined by the method of Turner (Turner, 1981). Measurement of solute leakage followed the method of Picchioni *et al.* (1991). Proline contents were determined according to Bates *et al.* (1973). The glycine betaine contents in plant tissues were determined according to Di Martino (2003) method. Chlorophyll contents were determined according to the method of Lichtenthaler (1987). To analyze of data, SAS software was used and Duncan's method at 5% level was used to compare the means of data.

Results

Stem and root length

According to the results of analysis of variance, it was found that the effect of salicylic acid on stem length was significant (Table 1). Applying 0.5mM and 1mM salicylic acid resulted in 10 and 8% increases in the stem length compared to control, respectively. The interaction effect showed that the highest mean stem length was referred to non-stress treatment, 0.5mM salicylic acid and all treatments in ascorbic acid. According to the results of analysis of variance, the effect of drought stress on the root length was significant at 5% level. Furthermore, it was identified that, the effects of salicylic acid and ascorbic acid were also significant at 5% level. Applying the levels of 60% and 30% usable moisture content, respectively resulted in 4% and 18% reductions in root length compared to control. The interaction effects showed that the highest mean root length belonged to the control and 0.5mM ascorbic acid.

Number of leaves and stem diameter

According to the results of variance analysis, it was identified that the effect of drought stress and salicylic acid on the number of leaves was significant at 5% level (Table 1). It was also identified that, the effects of drought stress on 1% salicylic acid was significant (Table 1) and moderate and severe stress respectively, resulted in 3% and 18% reductions in the number of leaves compared to control. Furthermore, applying the 0.5mM and 1.0mM salicylic acid treatments resulted in 23% and 5% increases in the number of leaves, respectively, in compare to control. The interaction effects showed that the highest mean number of leaves was belonged to non-stress moisture treatment with 0.5mM salicylic acid and 0.5mM ascorbic acid. According to the results of variance analysis, it was found that the drought stress in 1% salicylic acid on the stem diameter were also significant at 1% level (Table 1). It was found that treatment 30% of usable moisture content resulted in 8% reduction in stem diameter compared to control, whereas treatments 0.5mM and 1.0mM salicylic acid led to 12% and 6% reductions, respectively, in the stem diameter compared to control. The interaction effects showed that the highest mean of the stem diameter was belonged to control, salicylic acid, and 1mM ascorbic acid.

Leaf thickness and stem fresh weight

According to the results of analysis of variance, it was found that the effects of salicylic acid and ascorbic acid on leaf thickness were significant at levels 5% and 1%, respectively (Table 1). It was also identified that the drought stress in salicylic acid, and drought stress in ascorbic acid were significant at level 5% (Table 1), and the interactions effects between treatments on leaf thickness were also significant at level 5% (Table 1). Salicylic acid treatments 0.5mM and 1.0mM increased leaf thickness 1% compared to control. Similar results were also obtained in ascorbic acid 0.5mM and 1.0mM treatments. The interaction effects showed that the highest mean leaf thickness was referred to control, 1.0mM salicylic acid and 1.0mM ascorbic acid. According to the results of analysis of variance, it was found that the treatments did not have any significant effect on this trait.

Root and leaf fresh weight

According to the results of analysis of variance, it was found that the effects of stress factor on root fresh weight was significant at level of 1%. Furthermore, salicylic acid factor on root fresh weight was significant at level of 5%, respectively (Table 1). Applying the levels of 60% and 30% of usable moisture content, separately resulted in 2% and 33% reductions in the root fresh weight compared to control, whereas applying the treatment with 1mM salicylic acid resulted an increase (3%) in the root fresh weight compared to control. Based on the results of analysis of variance, it was identified that the effect of stress factor on the leaf fresh weight was significant at level 5% (Table 1). Also, applying the levels of 30% of usable moisture content resulted in a 21% reduction in the leaf fresh weight compared to control.

Root and leaf dry weight

According to the results of analysis of variance, it was found that, the effect of salicylic acid on the root dry weight was significant at levels 1% (Table 1). Using 0.5mM and 1.0mM of salicylic acid, separately, resulted in 5% and 33% increases in root dry weight respectively, compared to control. Treatment with 1mM ascorbic acid resulted in an increase (8%) in the root dry weight compared to control. According to the analysis of variance, it was found leaf dry weight was significantly affected by stress factor at level of 5% (Table 1). It was also found that applying the level of 30% usable moisture content led to a 19% reduction in leaf dry weight compared to control.

Photosynthetic pigments

According to the results of variance analysis, it was found that the effect of drought stress and salicylic acid on chlorophyll a was significant at level 1% (Table 1 and Table 2). Moreover, it was found that applying the levels of 60% and 30% usable moisture content resulted in 34% reduction in chlorophyll a content in compare to control. Additionally, 0.5mM and 1mM salicylic acid treatments resulted in 37.5% and 45% improvement in chlorophyll a content compared to control. According to the results of analysis of variance, it was found that the effect of drought stress and salicylic acid on chlorophyll b were significant at level 1% (Table 1). Applying the levels of 60% and 30% of usable moisture content resulted in 57% and 77% reductions in chlorophyll b compared to control, respectively. The highest mean of chlorophyll b was belonged to control (Table 2). Results of analysis of variance showed that the effect of stress factor and salicylic acid on total chlorophyll were significant at level 1% (Table 1 and Table 2). The obtained results indicated that the highest mean of total chlorophyll was belonged to control and 1mM salicylic acid.

Relative water content (RWC) and electrolyte leakage

The results revealed that the effect of stress factor and salicylic acid on relative water content was significant at levels 5% and 1%, respectively(Table 1). Also, applying the level of 30% usable moisture content resulted in a 7% reduction of the relative water content compared to control (Table 2). Moreover, 0.5mM and 1.0mM salicylic acid treatments caused in 14% and 15% increases in relative water content, respectively, compared to control. Results indicated that the highest mean of relative water content was belonged to control, 1mM salicylic and 1mM ascorbic acid (Table 2). It was shown that, the effect of stress factor and salicylic acid on electrolyte leakage were significant at levels 1% and 5%(Table 1). It was also identified that,applying the levels of 60% and 30% of usable moisture content resulted in 6% and 7% increases in electrolyte leakage compared to control (Table 2).

Proline and glycine betaine

The results of variance analysis showed that the effect of stress drought on proline content was significant at level 1% (Table 1). It was also identified that applying the levels of 60% and 30% usable moisture content resulted in 18% and 95% increases in proline content compared to control (Table 2). It was revealed that the highest mean of proline content was belonged to the treatment with 30% usable moisture content (Table 2). Results of the variance analysis indicated that the effect of stress factor on glycine betaine content was significant at level 1% (Table 1). Furthermore, applying the level of 30% usable moisture content resulted in an increase (18%) in glycine betaine content compared to control (Table 2).

Table 1.The mean square of the study traits in response to the experimental treatments of pistachio seedlings under drought stress conditions.

Source of Variations	df	Stem length	Stem diameter	Stem fresh weight	Stem dry weight	Root length	Root fresh weight	Root dry weight	Number of leaves	Leaf thickness	Leaf fresh weight	Leaf dry weight	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Relative water content	Electrolyte Leakage	Proline content	Glycinebetaine content
stress	2	11.1	1.4**	0.185	0.034	372*	0.285**	0.006	65.4*	0.002*	0.758*	0.057*	0.011**	0.027**	0.074**	301.9*	0.034**	74838.6**	66692.6**
salicylic acid	2	63.1*	1.3**	0.02	0.01	122.4	0.146*	0.066**	65.2*	0.003*	0.222	0.005	0.012**	0.007**	0.037**	1075.3**	0.018*	1293.8	3330.8
stress× salicylic acid	4	21.7	0.98**	0.035	0.017	37.6	0.032	0.007	51.8*	0.009**	0.15	0.013	0.011**	0.005**	0.03**	298.3*	0.012	1627.8	6581.2
ascorbic acid	2	19.4	0.007	0.026	0.013	32.1	0.009	0.044*	2.8	0.005**	0.08	0.005	0.0001	0.0005	0.001	3.6	0.009	553.1	757.3
stress× ascorbic acid	4	22.7	0.075	0.029	0.004	90.8	0.017	0.004	7.2	0.002*	0.029	0.004	0.00025	0.0002	0.00025	46.9	0.002	212.9	1280.1
salicylic acid × ascorbic acid	4	2.1	0.238	0.088	0.022	317.3*	0.141*	0.006	4.9	0.0005	0.16	0.02	0.001*	0.00075	0.003*	184.6	0.008	829.9	3236.9
stress × salicylic acid × ascorbic acid	8	8.2	0.211	0.064	0.012	89.3	0.027	0.015	17.5	0.001*	0.08	0.007	0.000375	0.000375	0.001	131.2	0.005	927.5	1590.1
error	80	19.0	0.272	0.066	0.015	120.1	0.047	0.014	14.6	0.001	0.081	0.012	0.000321	0.000432	0.001	92.2	0.005	1672.1	9159.5
Coefficients of variation (%)		17	16	22.8	23	24	23.8	24	21	14	19	22	24	22	21	13.9	9.25	24	21

ns, * and ** are non-significant, significant, at the probability of 5% and 1%, respectively.

Table 2. Means comparison of salicylic acid and ascorbic acid on morpho-physiological traits of pistachio seedlings under drought stress conditions.

Treatments	Stem length (cm)	Stem diameter (mm)	Stem fresh weight (g)	Stem dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Number of leaves	Leaf thickness (cm)	Leaf fresh weight (g)	Leaf dry weight (g)	Chlorophyll a ($mg.g^{-1}$.F.W.)	Chlorophyll b ($mg.g^{-1}$.F.W.)	Total Chlorophyll ($mg.g^{-1}$.F.W.)	Relative water content (%)	Electrolyte Leakage (%)	Proline content ($\mu mol{\cdot}g^{-1}{\cdot}$F.W)	betaine content ($\mu mol{\cdot}g^{-1}{\cdot}$F.W
control (non-stress)	24.96 a	3.206 ab	1.05 a	0.43 a	31.25 a	0.48 a	0.22 a	13.22 a	0.183 a	1.09 a	0.37 a	0.09 a	0.09 a	0.18 a	68.38 a	0.75 b	70.2 c	424.4 b
Moderate stress	25.93 a	3.361 a	0.93 b	0.41 ab	30.14 a	0.48 a	0.20 a	12.92 a	0.177 a	1.02 a	0.36 a	0.06 b	0.04 b	0.10 b	67.21 a	0.81 a	83.5 b	431.2 b
Severe stress	24.98 a	2.967 b	0.93 b	0.37 b	25.80 b	0.33 b	0.20 a	10.75 b	0.169 ab	0.81 b	0.30 b	0.06 b	0.04 b	0.10 b	64.00 b	0.80 a	137.1 a	502.1 a
salicylic acid 0mM	23.81 b	3.367 a	0.95 a	0.41 ab	26.93 b	0.37 b	0.18 b	11.22 b	0.166 b	0.88 b	0.33 a	0.05 c	0.04 b	0.09 b	62.67 b	0.77 b	83.3 b	443.9 b
salicylic acid 0.5mM	26.36 a	2.981 b	0.96 a	0.39 b	30.08 a	0.45 a	0.19 ab	11.86 b	0.181 a	1.00 a	0.34 a	0.08 a	0.06 a	0.14 ab	71.55 a	0.79 ab	81.4 b	450.9 b
salicylic acid 1mM	25.69 ab	3.186 ab	0.99 a	0.42 a	30.17 a	0.47 a	0.24 a	13.81 a	0.182 a	1.03 a	0.35 a	0.09 a	0.07 a	0.16 a	72.63 a	0.82 a	79.2 b	462.9 b
ascorbic acid 0mM	24.51 a	3.193 a	0.95 a	0.40 ab	28.06 ab	0.41 a	0.18 b	12.03 a	0.168 ab	0.92 a	0.33 a	0.07 b	0.05 a	0.12 b	69.15 a	0.80 a	82.3 b	447.6 a
ascorbic acid 0.5mM	25.38 a	3.174 a	0.96 a	0.39 b	29.19 a	0.44 a	0.22 a	12.28 a	0.172 a	0.98 a	0.34 a	0.07 b	0.05 a	0.12 b	68.58 a	0.80 a	85.0 b	453.6 b
ascorbic acid 1mM	25.97 a	3.167 a	1.0 a	0.43 a	29.93 a	0.44 a	0.23 a	12.58 a	0.189 a	1.01 a	0.35 a	0.07 b	0.05 a	0.12 b	69.11 a	0.82 a	90.0 ab	456.6 b

Means that are shared at least in one letter have no significant difference in Duncan's multiple range tests at the probability level of 5%

Discussion

According to the obtained morphological results of stem and roots, it was found that applying salicylic acid and ascorbic acid led to an increase in length of stem and roots. Photosynthesis is an important process to maintain plant growth and development; it is well known that photosynthetic systems in higher plants are most sensitive to drought stress (Falk *et al.*, 1996). The stress factor decreased the height through reduced photosynthesis (Németh *et al.*, 2002; Malinowska *et al.*, 2018). In this regard, the relationship between the role of salicylic acid in the absorption and transmission of ions (Harper and Balke, 1981), the rate of photosynthesis, porous conductance and transpiration (Khan *et al.*, 2003) was mentioned, so that, it can be effective in the height of the plant and production of the dry matter as a result of increasing in photosynthetic process. The decrease in the height of plant is usually due to a decrease in the number of nodes as well as the length of internodes. This reduction may be due to the reduced photosynthesis, suppressed cell division and reduced cellular prolongation (Hanson and Hitz, 1982; Korres *et al.*, 2016). The obtained results of the number of leaves indicated that applying the levels of 60% and 30% usable moisture content respectively, resulted in 3% and 18% reductions in the number of leaves, whereas treatments with applying 0.5mM and 1.0mM salicylic acid resulted in 23% and 5% increases in the number of leaves. These findings are consistent with the results of the reports of others (Bastam *et al.*, 2013; Aroca, 2012; Behboudian *et al.*, 1986; Sepaskhah and Maftoun, 1982). Loka and Oosterhuis also reported that the numbers of cotton’s leaves are reduced in drought stress condition (Loka and Oosterhuis, 2014). The results of stem diameter showed that applying the level of 30% of usable moisture content resulted in an 8% reduction in stem diameter compared to control, whereas treatments with applying 0.5mM and 1.0mM salicylic acid led to 12% and 6% reductions These findings are consistent with the results of the reports of others (Bastam *et al.*, 2013; Aroca, 2012; Behboudian *et al.*, 1986; Sepaskhah and Maftoun, 1982). The obtained results indicated that the highest mean leaf thickness was belonged to control, 1.0mM salicylic acid and 1.0mM ascorbic acid. Drought stress leads to a decrease in leaf thickness through reduced photosynthesis (Németh *et al.*, 2002; Malinowska *et al.*, 2018). In this regard, the relationship between the role of salicylic acid in the absorption and transmission of ions (Harper and Balke, 1981), the rate of photosynthesis, porous conductance and transpiration (Khan *et al.*, 2003) was mentioned so that it can be effective in the leaf thickness of the plant and production of the dry matter through increasing photosynthesis. The decrease in the leaf thickness may be due to the reduced photosynthesis and suppressed cell division (Hanson and Hitz, 1982; Korres *et al.*, 2016). The data revealed that drought stress condition resulted in reductions of stem fresh weight, in addition applying all treatments of salicylic acid and ascorbic acid resulted in an increase of stem fresh weight. These results are consistent with the results of the findings of others (Bastam *et al.*, 2013; Aroca, 2012; Behboudian *et al.*, 1986; Sepaskhah and Maftoun, 1982). The obtained results indicated that reductions of root and leaf fresh weight in plants under stress, in addition, applying all treatments of salicylic acid and ascorbic acid resulted in increases of root and leaf weight. These results are consistent with the findings of others (Bastam *et al.*, 2013; Aroca, 2012; Behboudian et al., 1986; Sepaskhah and Maftoun, 1982). Shirani Bidabadi *et al.* (2012) reported that applying salicylic acid (3% concentration) on banana plant *in vitro* water stress condition led to improved leaf production, increased fresh weight and relative water content. Medrano *et al.* (2015) concluded that stressed treatments generally produce less dry matter compared to control. In a study conducted by Saeidi and Abdoli (2015) on wheat cultivars, a sharp decrease was seen in the weight of dry matter under drought stress condition compared to control (Saeidi and Abdoli, 2015). The findings of root dry weight indicated that applying 0.5mM and 1.0mM of salicylic acid, resulted in 5% and 33% increases in root dry weight. These results are consistent with the findings of others (Bastam *et al.*, 2013; Aroca, 2012; Behboudian et al., 1986; Sepaskhah and Maftoun, 1982). Bijanzadeh *et al.* (2019) discussed that drought stress reduces the weight of the maize plant and using salicylic acid mitigates the effects of the stress and increases the root dry weight. On the other hand, treatment with 1mM ascorbic acid resulted in an increase (8%) in root dry weight. These results are consistent with the findings of others (Xu *et al.*, 2015). The obtained results showed reductions of leaf dry weight in plants under stress. Applying the level of 30% usable moisture content led to a 19% reduction in leaf dry weight. In addition, applying all treatments of salicylic acid and ascorbic acid increased leaf dry weight. These results are consistent with the findings of others (Bastam et al., 2013; Aroca, 2012; Behboudian et al., 1986; Sepaskhah and Maftoun, 1982). Hanson and Hitz (1982) also stated that the drought stress results in the reduced plant dry matter. In this experiment, it was also found that the chlorophyll contents in leaves were affected significantly in low water conditions, so that, the highest mean of chlorophyll a was belonged to control, 0.5mM salicylic acid and 0.5mM ascorbic acid. In addition, the highest mean of chlorophyll b was belonged to control, 1mM salicylic acid and 1mM ascorbic acid. The highest mean of total chlorophyll was belonged to control, 0.5mM salicylic acid and 0.5mM ascorbic acid. These results are consistent with the findings of others (Sedaghati and Hokmabadi, 2015; Bastam *et al.*, 2013; Aroca, 2012; Ebrahimian and Bybordi, 2012). It could be concluded that drought stress decreased chlorophyll contents sharply. Significant decrease in chlorophyll, carotenoid, and total pigments under drought stress is partly due to water shortage and mainly due to damage to chloroplasts caused by active oxygen species (Fahad *et al.*, 2017). It was reported that salicylic acid and ascorbic acid plays a vital role in increasing the amount of chlorophyll and relative water content (Nazar *et al*., 2015; Bastam *et al*., 2013; Ebrahimian and Bybordi, 2012). The obtained results indicated that applying the low water conditions significantly affected on the relative water content, so that, the highest mean of relative water content was belonged to treatment without drought stress, 1mM salicylic and 1mM ascorbic acid. These results are consistent with the findings of others (Hasanuzzaman *et al.*, 2019; Razmi *et al.*, 2017; Sedaghati and Hokmabadi, 2015; Bastam *et al.*, 2013). They all reported that applying salicylic acid and ascorbic acid led to improved relative water content. It was found that,applying the low water conditions significantly affected on electrolyte leakage, so that, the highest mean of electrolyte leakage was belonged to the stress treatment and without applying salicylic acid and ascorbic acid. (Ahanger *et al.*, 2017; Khan *et al.*, 2015; Manzoor *et al.*, 2015; Zonouri *et al.*, 2014). The results showed that applying the low water conditions significantly affected on proline content in leaves, so that, the highest mean of proline content was belonged to the treatment with 30% usable moisture content, 1mM salicylic acid, and 0.5mM ascorbic acid. The higher accumulation of the proline content noticed in drought stresses plants indicates an effective adjustment and adaptation to drought conditions which are supported by other investigations (Elhakem, 2019; Chun *et al.*, 2018; Penella *et al.*, 2017; Bastam *et al.*, 2013; Lotfi *et al.*, 2010). The rising in proline levels could be from the metabolic responses which associate the recognition of stress condition to the induction of physiological reactions (Liang *et al.*, 2013). The results showed that applying the low water condition significantly affected on glycine betaine content, so that, the highest mean of glycine betaine content was belonged to the treatment with 30% usable moisture content. The higher accumulation of the glycine betaine content plants under abiotic stresses indicates an effective adjustment and adaptation to drought conditions which are supported by others (Hasanuzzaman *et al.*, 2019). Many higher plants naturally accumulate glycine betaine as a protectant under stress condition and acting as an osmoprotectants in stressed plants (Aroca, 2012).

Conclusions

The results of this study showed that drought stress had negative effect on pistachio seedlings growth. Base on the results, it can be concluded that foliar application of salicylic acid and ascorbic acid improved pistachio morpho-physiological responses to drought conditions. Foliar application of 0.5mM salicylic acid, 0.5mM and 1mM ascorbic acid were more effective than other treatments to improve the adaptation of pistachio seedlings to moderate drought stress. Thus, the recommended concentrations of salicylic acid and ascorbic acid were accompanied with some physiological processes such as enhancing plant growth indicators, chlorophyll (a, b or total), relative water content and proline content. Moreover, these findings indicate that salicylic acid and ascorbic acid could be used to improve the pistachio seedlings growth and development under drought stress.

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