

## Improved growth indices and tolerance of myrtle (*Myrtus communis* L.) to water-deficit stress by alleviating antioxidants and compatible osmolytes using a superabsorbent polymer

Somayeh Esmaeili\*, Abbas Danaifar

Department of Horticultural Science, School of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran

 [s.esmaeili@scu.ac.ir](mailto:s.esmaeili@scu.ac.ir)

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### Abstract

Superabsorbent polymers (SAPs) are able to increase soil moisture and improve plant growth. A greenhouse experiment was conducted to investigate the interaction between different irrigation levels (100% field capacity (FC), 75% FC, and 50% FC) and A200-SAP (0, 1, and 2 g kg<sup>-1</sup> dry soil weight) in 1-year old myrtle plants. The results showed that water-deficit treatment (50% FC) significantly reduced growth indices and also increased proline, total phenol, and the activities of antioxidant enzymes compared to well-watered plants (100% FC). While, total soluble protein, flavonoids, and total antioxidant capacity (TAC) had no significant change by increasing water-deficit stress compared to control, added SAP (2 g kg<sup>-1</sup>) led to an increase of about 43.81% in root length compared to non-SAP treatment at 50% FC. Furthermore, the use of SAP (2 g kg<sup>-1</sup>) significantly increased the content of proline and the activities of antioxidant enzymes, especially catalase (CAT), under water-deficient conditions by providing more water and reducing reactive oxygen species. The results indicated that young myrtle plants are highly adapted to adverse environmental conditions by increasing antioxidant compounds and osmoregulators. Moreover, they could produce new shoot primordia when SAP is added, in both well-water and water-deficient treatments. Therefore, SAPs can be applied in a cost-efficient and efficient manner to the roots of young woody plants to increase their survival and performance efficiency.

**Keywords:** Ascorbate peroxidase, Catalase, Phenol, Proline, Total antioxidant capacity.

### Introduction

The myrtle plant (*Myrtus communis* L.) is distributed in arid and semi-arid regions of Iran. This plant has very important in beautifying the environment, developing urban and suburban green spaces, restoring natural ecosystems, and medicinal uses (Salehnia, 2008). The optimization of the utilization of water resources is strategic for the long-term competitiveness of the agricultural industry in the world. There will be a major challenge in water management in the near future; by 2030, water demand is expected to increase by 50 % and withdrawals could exceed natural renewal by 60 %, causing water scarcity (Saguy *et al.*, 2013; Nestlé, 2011).

The plant's response to water-deficit stress is complex and involves changes at all levels of morphology, physiology, metabolism, and molecular. A reduction in the growth of plants is one of the most common symptoms of drought stress (Hussain *et al.*, 2018; Sairam & Srivastava, 2001). In general, plant responses depend on the severity and duration of stress, plant species, developmental stage, and their interactions with environmental factors. It is difficult to say exactly which of these factors causes water-deficit tolerance in plants. Therefore, understanding the plant responses to these stresses is the key factor for the improvement of their tolerance and yield.

In plants exposed to stress conditions, proline acts as an excellent osmolyte, it is also a metal chelator, an antioxidant defense molecule, and a signaling molecule. Previous studies have shown that plants overproduce proline in stressful environments, which in turn increases stress tolerance by maintaining turgor or osmotic balance and stabilizing membranes. Plants are also prevented from experiencing

oxidative bursts by preventing electrolyte leakage and reducing reactive oxygen species (ROS) concentrations (Ashraf & Foolad 2007; Hayat *et al.*, 2012).

SAPs are highly water-absorbent and have high water-storage capabilities (more than 400-1500 times per dry weight). They can increase soil moisture and help plants to grow in water-deficient environments. Several researches have indicated that SAPs can increase plant growth, survival, water-use efficiency, and dry matter production by increasing nutrient efficiency and maintaining soil moisture (Azevedo *et al.*, 2016; Tomášková *et al.*, 2020; Patra *et al.*, 2022).

Khaleghi & Moallemi (2018) stated that water deficit could affect directly cell turgor potential and reduce cell division, cell enlargement, photosynthesis, and plant growth of two olive cultivars, 'Baghmalek' and 'Dezphol'. The application of 3 g kg<sup>-1</sup> of A200-SAP showed the highest leaf area and leaf area ratio in 'Baghmalek' cultivar. In addition, root fresh and dry weights and plant height were 36, 49.7 and 27 % greater in 'Baghmalek' cultivar treated with 30% evapotranspiration (ETcrop) and 4 g kg<sup>-1</sup> SAP in comparison with 'Baghmalek' cultivar treated with 30% ETcrop and without SAP.

It has been reported by Li *et al.* (2018) that water stress inhibited the growth and development of young Areca seedlings (*Areca catechu* L.), and increased the activity of antioxidant enzymes, particularly peroxidase and superoxide dismutase. While, the use of SAPs caused a significant increase in plant weight, chlorophyll content, and photosynthesis under severe water stress.

Kenawy *et al.* (2018) reported that water stress caused a significant reduction in the growth and yield of rice (*Oryza sativa* L. cv. Giza 177), maize (*Zea mays* L. cv. tri-hybrid 311) and peanut (*Arachis hypogaea* L. cv. Giza 5) plants. Ion leakage, malondialdehyde, free sugar, amino acid, proline, glycine betaine, phenolic, and flavonoids under drought stress were increased whereas the use of SAP improved growth, yield, compatible osmolytes, and antioxidants.

This study aimed to investigate the effects SAPs on improving the growth of young myrtle plants and increasing tolerance to water-deficit stress by activating antioxidant mechanisms and compatible osmolytes.

## Materials and Methods

### Plant materials and experimental conditions

The one-year-old common myrtle plants derived from stem cuttings were used in the present study. This research was done at the Research Greenhouse of the Department of Horticultural Science, School of Agriculture, Shahid Chamran University, Ahvaz, Iran, from March to July 2021. The soil mixture consisted of a 4: 2: 1 ratio of soil, rotted cow manure, and sand in pots with a top diameter of 28 cm and height of 24 cm. A200-SAP (Iranian Nano Arian Company) was applied according to the manufacturer's instructions by mixing with some potting soil and placed around the roots. Cultured plants were irrigated once every two days until completely established.

The experiment treatments included three levels of irrigation (100%, 75%, and 50% FC) and three levels of A200 -SAP (0, 1, and 2 gr kg<sup>-1</sup> dry soil) with four replications. Irrigation treatments were calculated based on field capacity (FC) and permanent wilting point (PWP), which were 33% and 20%, respectively. Soil mixture properties, including soil texture (silty loam), organic matter percentage (3.8%), bulk density (1.16 gr cm<sup>-3</sup>), EC (4 mmhos cm<sup>-1</sup>), and pH (7.3) measured before the start of the test. Mean temperature and light intensity during the experiment were 28±2 °C and 5700 lux, respectively.

### Growth parameters

Shoot and root lengths were measured with a millimeter ruler after separating the shoot from the root. The shoot and root fresh weights of samples were measured using an electronic balance. After that, the samples were placed in the oven for 48 h at a temperature of 70 °C for measuring dry weights.

### Biochemical analysis

Proline content was assayed by the method of Bates *et al.* (1973). Briefly, 0.5 g of fresh leaf sample was homogenized with 5 mL of 95% ethanol in a porcelain mortar. Then, 2 mL of the prepared alcoholic extract was poured into 15 mL test tubes and 2 mL of ninhydrin and 2 ml of glacial acetic acid were added. Then, the solution was placed in a boiling water bath for 1 h and transferred to an ice water container to stop the amino acid reaction with ninhydrin. After cooling, 4 mL of toluene was added to each test tube and completely mixed using a vortex for 15-20 s. The upper phase absorption of the samples was read at 520 nm using a spectrophotometer (7315 model, Jenway, UK).



The percentage of antioxidant activity of each sample was measured by DPPH free radical assay according to the method described by Sun *et al.* (2007). The samples were reacted with the stable DPPH radical in an 80% methanol solution. The reaction mixture consisted of 1 mL of extract and 1 mL of solution (100 mL of 80% methanol and 0.008 g of DPPH). The changes in color (from deep violet to light yellow) were read at 517 nm after 30 min at dark using a spectrophotometer (UV-2100 model, USA). The mixture of 80% methanol (1 mL) and DPPH radical solution (1 mL) serve as a blank. The TAC was estimated using the following formula:

$$\text{TAC (\%)} = (\text{A}_{\text{control}} - \text{A}_{\text{sample}}) / (\text{A}_{\text{control}}) \times 100$$

Where:  $\text{A}_{\text{control}}$  is the absorbance of the control (no sample, DPPH solution only) and  $\text{A}_{\text{sample}}$  is the absorbance in the presence of the sample.

Leaf total flavonoid content was measured based on the method of Chang *et al.* (2002). Briefly, 1 g of fresh leaf with 10 mL of 80% methanol was homogenized. Then, 125  $\mu$ L of the extract was dissolved in 1.5 mL methanol, and 0.1 mL 10% aluminum chloride was added. Then, 0.1 mL of one molar potassium acetate solution and 2.8 mL of distilled water were added to the mixture and kept at room temperature for 30 min. Quercetin (Q) was used to draw the standard curve. The absorbance of the mixture was measured at 415 nm with a spectrophotometer (UV-2100 model, USA).

Total phenol was measured as described by McDonald *et al.* (2001). Briefly, 100  $\mu$ L of the extract was diluted with 1 ml of 80% methanol. Then, 2.5 mL of 10% Folin-Ciocalteu's phenol reagent was added to 250  $\mu$ L of diluted extract, and was added 2 mL of 1 M sodium carbonate to each of the samples. Then, the samples were placed in a warm water bath (45 °C) for 15 min. Standard solutions were prepared of different concentrations of gallic acid (GA) (0, 50, 100, 150, 200, and 250 mg L<sup>-1</sup>) with a ratio 1:1 of methanol and distilled water. Absorbance was read at 765 nm using a spectrophotometer (UV-2100 model, USA).

Total soluble protein was measured by the method of Bradford (1976). Catalase (CAT), guaiacol peroxidase (POD), and Ascorbate peroxidase (APX) enzymes activity were assayed according to the method of Beers & Sizer (1952), Hemeda & Klein (1990) and Nakano & Asada (1981), respectively.

### Experimental design and data analysis

Treatments were arranged in a factorial experiment in a completely randomized design with four replications. Data were analyzed using SAS 9.4 Software, and means were compared using the Duncan test at  $p \leq 0.05$ .

## Results and Discussion

### Growth responses

Water treatments significantly affected growth parameters, including shoot and root length, and fresh and dry weight of shoots and roots (Table 1). The highest shoot length was observed in 75% FC. In non-SAP treatment, there was a significant increase of 27.17% in shoot length at 75% FC compared to 100% FC. While, root length decreased by about 27.49%, and fresh and dry weights of roots increased by about 9.51% and 12.23% at 75% FC compared to 100% FC, respectively (Table 1). Shoot fresh and dry weight showed a significant reduction with increasing water-deficit stress. The highest shoot fresh weight was found in 75% FC, and the lowest was obtained in 50% FC and 2 g kg<sup>-1</sup> soil of SAP. As shown in Table 1, water stress significantly reduced root dry weight in 50% FC compared to the control treatment (100% FC). The highest root dry weight was observed in 100% FC and 2 g kg<sup>-1</sup> soil of SAP. Cell growth is one of the most sensitive physiological processes that severely affected by water stress when turgor pressure decreases (Anjum *et al.*, 2011). According to the results, water-deficit stresses had adverse effects on appearance and growth parameters. Despite SAP treatments, plants increased shoot growth and were healthier under low-water stress (75% FC) and non-stress conditions. Plants exposed to moderate water stress (50% FC) showed leaf rolling, browning, and falling. However, the SAP application improved visual quality, and shoot fresh and dry weights of young myrtle plants under well-watered and water-deficit stress conditions. These results indicated that common myrtle had a high potential adaptation to adverse environments. Another possible reason for increased biomass can be attributed to proper aeration under 75% and 100% FC with SAP, which led to production of new growth of shoot primordia under well-watered and water-deficit stress conditions.

Shoot fresh and dry weights were reduced by 61.75% and 51.73 % at 50% FC, respectively, compared to 100% FC. Additionally, 2 g kg<sup>-1</sup> of SAP had an increase of about 21.21% compared to non-SAP treatment (Table 1). These results indicated that the response of myrtle plants varies with stress severity.



Application of SAP ( $2\text{ g kg}^{-1}$  soil) increased root dry weight compared to control up to 20.28% in well-watered plants. Generally, SAP levels did not show significant differences in growth parameters at 50% FC. However, some traits such as root length, shoot and root fresh, and dry weights partially increased. Applying SAP at  $2\text{ g kg}^{-1}$  soil significantly showed an increase of about 43.81% in root length compared to non-SAP treatment at 50% FC.

**Table 1. Effect of different irrigation levels and SAP (Super Absorbent Polymer), and their interaction on some growth responses of *M. communis*.**

Variables	SAP ( $\text{g kg}^{-1}$ soil)	Irrigation levels			Mean
		100% FC	75% FC	50% FC	
Shoot length (cm)	0	46 $\pm$ 3.24 bc	58.50 $\pm$ 2.59 a	38.5 $\pm$ 2.10 cd	47.66 $\pm$ 2.85 A
	1	54 $\pm$ 3.46 ab	40.25 $\pm$ 2.62 cd	41.00 $\pm$ 3.76 cd	45.08 $\pm$ 2.57 AB
	2	51.5 $\pm$ 4.17 ab	40.00 $\pm$ 3.00 cd	35.00 $\pm$ 2.41 d	42.16 $\pm$ 2.69 B
	Mean	50.50 $\pm$ 2.15 A	46.25 $\pm$ 2.98 A	38.16 $\pm$ 1.66 B	
Shoot fresh weight (g)	0	33.62 $\pm$ 1.09 b	33.85 $\pm$ 1.45 b	15.56 $\pm$ 0.54 e	27.67 $\pm$ 2.60 B
	1	49.69 $\pm$ 0.51 a	27.62 $\pm$ 1.10 c	17.25 $\pm$ 0.85 e	31.46 $\pm$ 4.08 A
	2	46.69 $\pm$ 0.94 a	22.66 $\pm$ 1.25 d	16.86 $\pm$ 0.95 e	28.74 $\pm$ 3.93 B
	Mean	43.27 $\pm$ 2.13 A	28.04 $\pm$ 1.53 B	16.55 $\pm$ 0.47 C	
Shoot dry weight (g)	0	15.21 $\pm$ 0.41 b	14.00 $\pm$ 0.46 b	8.55 $\pm$ 0.26 d	12.59 $\pm$ 0.89 B
	1	25.02 $\pm$ 1.02 a	13.49 $\pm$ 0.25 b	10.05 $\pm$ 0.55 cd	16.18 $\pm$ 1.96 A
	2	23.67 $\pm$ 1.35 a	11.12 $\pm$ 0.91 c	10.98 $\pm$ 0.36 c	15.26 $\pm$ 1.86 A
	Mean	21.30 $\pm$ 1.14 A	12.87 $\pm$ 0.49 B	9.86 $\pm$ 0.36 C	
Root length (cm)	0	42.75 $\pm$ 2.71 a	31.00 $\pm$ 1.00 cd	26.25 $\pm$ 1.31 d	33.33 $\pm$ 2.30 B
	1	42.00 $\pm$ 2.34 a	34.00 $\pm$ 2.12 bc	33.75 $\pm$ 1.49 bc	36.58 $\pm$ 1.56 AB
	2	41.50 $\pm$ 2.32 a	38.00 $\pm$ 1.87 ab	37.75 $\pm$ 2.28 ab	39.08 $\pm$ 1.24 AB
	Mean	42.08 $\pm$ 1.29 A	34.33 $\pm$ 1.25 B	32.58 $\pm$ 1.70 B	
Root fresh weight (g)	0	27.24 $\pm$ 2.38 ab	29.83 $\pm$ 4.83 ab	12.76 $\pm$ 1.25 c	23.28 $\pm$ 2.81 A
	1	26.65 $\pm$ 6.17 ab	22.77 $\pm$ 5.1 abc	17.03 $\pm$ 4.29 bc	22.15 $\pm$ 2.98 A
	2	33.14 $\pm$ 3.28 a	27.38 $\pm$ 3.09 ab	17.56 $\pm$ 2.49 bc	26.03 $\pm$ 2.48 A
	Mean	29.01 $\pm$ 2.39 A	26.66 $\pm$ 2.47 A	15.79 $\pm$ 1.67 B	
Root dry weight (g)	0	12.92 $\pm$ 0.90 ab	14.5 $\pm$ 2.43 ab	6.70 $\pm$ 0.75 c	11.37 $\pm$ 1.30 A
	1	12.72 $\pm$ 2.54 ab	11.79 $\pm$ 2.43 abc	8.65 $\pm$ 1.67 bc	11.05 $\pm$ 1.28 A
	2	15.54 $\pm$ 1.69 a	13.80 $\pm$ 1.40 ab	9.32 $\pm$ 1.14 bc	12.89 $\pm$ 1.08 A
	Mean	13.73 $\pm$ 1.03 A	13.36 $\pm$ 1.17 A	8.22 $\pm$ 0.73 B	

In each variable, data followed by the same letters  $\pm$  SE (small letters for interactions and capital letters for means of rows and columns) are not significantly different using Duncan test at 5% level.

These results suggested that applying even a minimal amount of SAP ( $1\text{ g kg}^{-1}$  soil) can improve the growth characteristics of the young myrtle plants under well-watered and water-deficit stresses.

Similar results were obtained by adding SAP on growth parameters in some young woody plants, including *Cupressus arizonica* (Abedi-Koupai & Asadkazemi, 2006), Citrus (Arbona *et al.*, 2005), *Eucalyptus* sp. (Khodadadi-Dehkordi, 2017), and *Populus* spp. (Beniwal *et al.*, 2010; Shi *et al.*, 2010), and *Ficus benjamina* L. 'Starlight' (Ghasemi Ghehsareh *et al.*, 2010) under water stress conditions. However, Liu *et al.* (2018) stated that SAP negatively affected the seedling growth of *Areca catechu* L. under full irrigation conditions, contrary to our findings. According to Kargar *et al.* (2017), SAPs may affect specific species, explaining the different results.

Furthermore, Kenway *et al.* (2018) stated that drought stress decreased rice, maize, and peanut growth, which led to reduced stem height and leaf area. While SAP (0.5% w/w) application enhanced the growth of crops in both well-watered and drought stress conditions. The benefits of SAP on morphological traits may be due to increased soil water storage capacity, increased water and nutrient uptake by plants, increased levels of PGRs, especially auxins, and decreased ROS levels (Kargar *et al.*, 2017; Mazloom *et al.*, 2020).



### Biochemical responses

Despite SAP treatment, proline content significantly increased at 50% FC compared to well-watered plants (100% FC). Results showed that added SAP decreased proline content in all irrigation levels; however, it decreased more in well-watered plants. In addition, the application of SAP had no significant difference between 75% and 100% FC on proline content, while, it was significant under non-SAP treatment. The highest and lowest proline content were found in 50% FC without SAP treatment and 100% FC with 2 g kg<sup>-1</sup> SAP, respectively (Table 2). Added SAP could reduce proline content in all irrigation levels; however, this reduction was more (about 17.86%) in well-watered plants compared to non-SAP treatment (Table 2). There are conflicting results from the effect of SAPs on proline content under water-deficit stresses. It was reported that SAP had an increasing effect on proline content (Tomáškov *et al.*, 2020), while in others, there were opposite results (Najafinezhad *et al.*, 2014; Kenway *et al.*, 2018; Başak, 2020).

Studies have shown that environmental stresses lead to proline accumulation in plants, which contributes to increased tolerance of plants through the rise in osmotic adjustment, cellular turgor, and integrity of membranes to prevent electrolyte leakage and reduce oxidative burst by bringing ROS concentrations within normal ranges (Kenway *et al.*, 2018). In addition, proline acts as a nitrogen reservoir and enzymes stabilizer under stress conditions (Farooq *et al.*, 2017). Also, the breakdown of proline upon stress may provide adequate reducing agents for mitochondrial oxidative phosphorylation and the production of ATP during stress recovery (Hare *et al.*, 1998). Similar to the present results, the use of SAP in the rhizosphere reduced proline accumulation in some crops including sunflower (Nazarli *et al.*, 2010) and sweet pepper (Sayyari & Ghanbari, 2012).

As shown in Table 2, SAP treatments were not significantly different in TAC content under 100% FC and 50% FC. Adding 2 g kg<sup>-1</sup> soil of SAP led to a slight increase in TAC amount in more treatments. However, a significant reduction of about 4.30% of TAC was observed under 75% FC and 1 g kg<sup>-1</sup> soil of SAP compared to 100% FC.

Table of variance analysis of flavonoids showed the main effects of irrigation levels were significant at the 5% level (Data not shown). However, the main effects of SAP treatments and their interaction with irrigation levels were not significant. There was a slight decrease of about 5.39% in total flavonoids at 75% FC compared to 100% FC but reached the level of control plants at 50% FC (Table 2).

Total phenol significantly decreased with reducing irrigation level to 50% FC (Table 2). In non-SAP treatment, a reduction of about 28.61% was found at 50% FC compared to 100% FC. Applying 1 g kg<sup>-1</sup> soil of SAP did not change the total phenol content in 75% FC and 100% FC treatments. While a remarkable increase of about 55.89% was found at 50% FC and 1 g kg<sup>-1</sup> SAP compared to the control treatment.

Total flavonoid and total antioxidant capacity under water-deficit stresses remained similar to control plants. However, total phenol significantly decreased at mid-water stress. These results are against Azizi *et al.* (2021), who stated that severe water stress led to a significant accumulation of phenolic, flavonoids, and antioxidant compounds in myrtle plants. These conflicting results could be due to differences in method, stress intensity, sampling time, origin, and age of plants. Another possible explanation for the reduction of total phenol is the increased use of carbohydrates to produce essential oils rather than phenols.

Based on the results, the leaves of the myrtle plants are potential sources of antioxidants and phenolic compounds, as found in previous studies (Gardeli *et al.*, 2008; Mohamadi *et al.*, 2021). In the present results, these components led to high antioxidant capacity in stress and non-stress treatments. The significant increase in phenolic compounds, flavonoids, and antioxidant capacity in plants can be due to the coincidence of sampling time with the flowering stage of plants. These results are consistent with previous reports, which show that the highest levels of these compounds are made in leaves during the flowering stage in late spring to mid-summer (Gardeli *et al.*, 2008; Mohamadi *et al.*, 2021). They also found a positive correlation between phenolic compounds, flavonoids, and antioxidant compounds.

**Table 2. Effect of different irrigation levels and SAP (Super Absorbent Polymer), and their interaction on biochemical responses of *M. communis*.**

Variable	SAP (g kg <sup>-1</sup> )	Irrigation levels			Mean
		100% FC	75% FC	50% FC	
Proline (μmol g <sup>-1</sup> f.w.)	0	21.39±1.03 bc	22.75±0.79 abc	25.06±0.45 a	23.07±0.61 A
	1	17.98±0.62 d	20.57±0.63 c	23.57±1.05 ab	20.70±0.80 B
	2	17.57±0.95 d	21.79±0.90 bc	23.22±0.45 ab	20.86±0.83 B
	Mean	18.98±0.69 C	21.70±0.49 B	23.95±0.44 A	
TAC (%)	0	92.03±0.93 abc	91.62±0.72 abc	90.46±0.29 cd	91.37±0.41 A
	1	93.46±0.11 a	89.44±1.42 d	90.46±0.33 cd	91.12±0.67 A
	2	93.32±0.23 ab	91.96±0.39 abc	91.28±0.27 bcd	92.18±0.30 A
	Mean	92.93±0.35 A	91.00±0.60 B	90.73±0.19 B	
Total flavonoid (mg Quercetin g <sup>-1</sup> )	0	118.75±1.01 a	113.50±1.43 a	118.75±6.63 a	117.00±2.20 A
	1	118.75±2.26 a	113.50±1.43 a	110.88±2.63 a	116.42±1.35 A
	2	119.63±0.88 a	110.88±0.88 a	118.75±1.75 a	114.38±1.50 A
	Mean	119.04±0.80 A	112.63±0.76 B	116.13±2.48 B	
Total phenol (mg GallicAcid g <sup>-1</sup> )	0	93.37±2.24 ab	90.92±4.28 ab	66.66±11.72 c	83.65±5.27 A
	1	64.82±3.26 c	62.36±8.13 c	101.05±3.02 a	76.08±6.02 A
	2	75.57±6.46 bc	79.56±4.32 bc	77.41±9.74 bc	77.51±3.79 A
	Mean	77.92±4.22 A	77.61±4.68 A	81.71±6.38 A	

In each variable, data followed by the same letters  $\pm$  SE (small letters for interactions and capital letters for means of rows and columns) are not significantly different using the Duncan test at the 5% level.

The amount of protein significantly decreased at 75% FC and 2 g kg<sup>-1</sup> soil of SAP compared to non-SAP treatment. However, no remarkable reduction was found between 100% FC and 50% FC by adding SAP (Fig. 1A).

Data analysis showed that the interaction of irrigation and SAP levels significantly affected POD activity. In non-SAP treatments, POD activity significantly increased in 50% FC compared to 100% FC (Fig. 1B). However, SAP had no significant effect on POD activity in control plants (100% FC). POD activity significantly increased compared to control plants by reducing irrigation levels up to 50% FC. Adding SAP could significantly decrease POD activity at 50% FC compared to the non-SAP treatment (Fig. 1B).

CAT activity increased by reducing the irrigation level to 50% FC under non-SAP treatment. The application of 2 g kg<sup>-1</sup> soil of SAP notably reduced the activity of this enzyme at 50% FC (Fig. 2C). Regardless of SAP treatment, APX activity notably increased with decreasing irrigation levels. The highest activity of the APX enzyme was observed at 50% FC and without SAP treatment. Added SAP decreased APX activity in all treatments. However, there was no significant difference between SAP and non-SAP treatments in other irrigation levels.

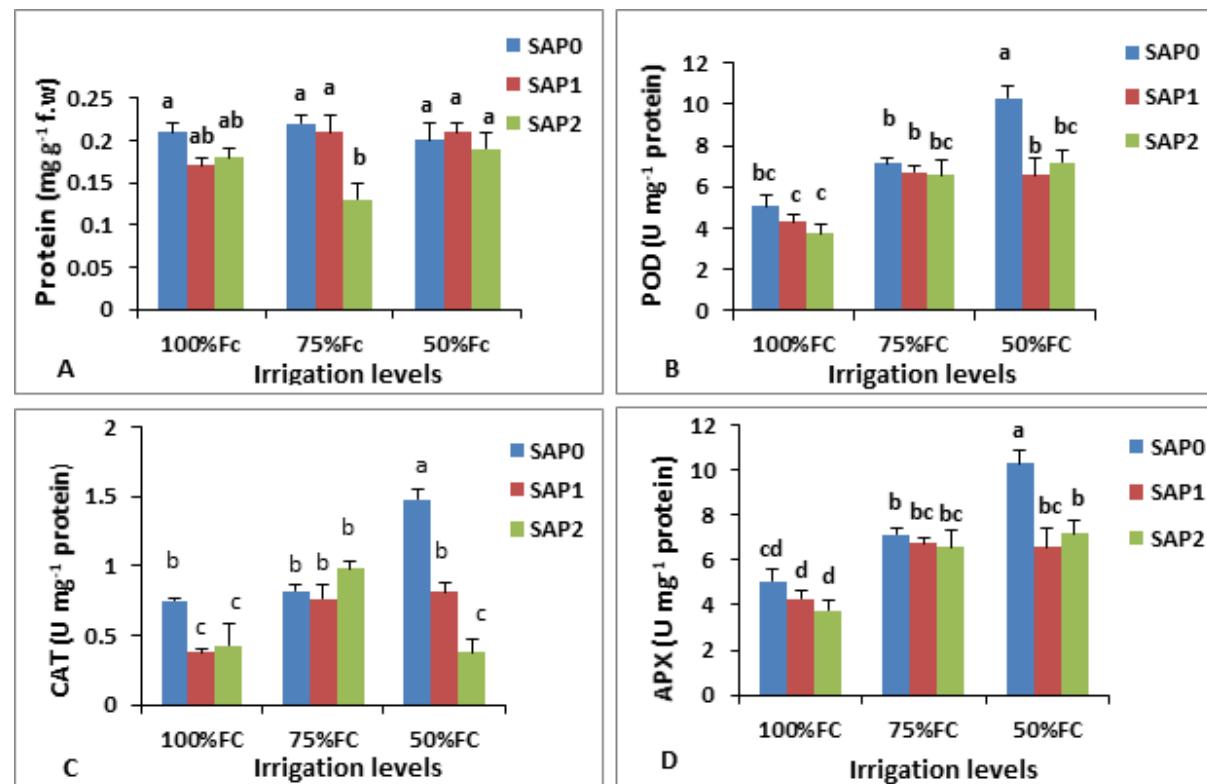
Soluble proteins partially increased at 75% FC and again decreased at 50% FC. While added SAP (2 g kg<sup>-1</sup> soil) significantly decreased soluble proteins under 75% FC. Reduced soluble proteins were associated with increased proline content under water-deficit stress. These findings are similar to the results reported by Kenway *et al.* (2018) on rice, maize, and peanut. They stated that drought led to a rapid depletion in soluble proteins associated with increasing proline and amino acids. These results are probably due to decreased protein synthesis and increased activity of proteolytic enzymes or oxidative damage.

Similar to previous reports, POD, CAT, and APX activities significantly increased with the progress of water stress compared to the control treatment (Figure 1A-D). However, SAP decreased antioxidant enzyme activities in both stress and non-stress conditions. Thus, 2 g kg<sup>-1</sup> soil of SAP could remarkably



reduce POD, CAT, and APX activities at 50 % FC. In addition, the same amount of SAP significantly declined CAT activity in non-stress plants.

It may be due to the positive effect of SAPs on increasing ventilation in the rhizosphere in non-stressed conditions, which improved growth conditions. Furthermore, SAP improved growth parameters by providing available water and scavenging ROSs under water-deficit stresses. It has been proved that efficient antioxidative characteristics can have better protection against oxidative stress in plants under water stress (Kenway *et al.*, 2018; Azizi *et al.*, 2021). It also reported that the ability of *Acacia victoriae* seedlings to tolerate water stress conditions is associated with an increase in CAT and POD activities in this species and is often accompanied by decreasing leaf area and increasing chlorophyll content. However, different levels of SAP improved growth characteristics and reduced the activity of CAT and POD enzymes (Tongo *et al.*, 2014).



**Figure 1. Interaction of irrigation and SAP (Super Absorbent Polymer) levels on protein content (A), POD (B), CAT (C), APX (D) activities. Distinct letters represent statistically significant differences by the Duncan test ( $P \leq 0.05$ ). Bars represent SE.**

## Conclusion

It can be concluded that tolerance of young myrtle plants was associated with increased proline and antioxidant enzyme activities under low and mid water-deficit stresses. In addition, leaf myrtle extracts had high natural amounts of phenolic components, flavonoids, and carotenoids in both well-watered and water-deficit stress, resulting in a high antioxidant capacity. A200-SAP improved growth conditions by providing more water and eliminating ROSs in stress and non-stress plants. Therefore, SAPs could act as a slow release of water within the soil and maintain young woody plants during extended periods of time. However, further research is needed to evaluate the effects of combining bio-additive material with higher levels of SAPs to improve myrtle young plants under severe water stresses.

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در مقاله های انگلیسی پیشین چکیده فارسی نداشتم فقط در وبگاه نیاز است.  
**بهبود شاخص های رشد و تحمل *Myrtus communis L.* به تنش کم آبی با کاهش آنتی اکسیدان ها و اسمولیت های سازگار توسط یک بسپار ابر جاذب**

سمیه اسماعیلی\*، عباس دانایی فر

بخش علوم باگبانی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران  
s.esmaeili@scu.ac.ir

**چکیده**

پلیمرهای ابر جاذب (SAPs) قادر به افزایش رطوبت خاک و بهبود رشد گیاه هستند. در این پژوهش، آزمایش گلخانه ای برای بررسی برهمکنش بین سطوح مختلف آبیاری (FC٪ ۱۰۰، ۷۵٪ FC و ۵۰٪ A200-SAP) و (FC٪ ۱۰، ۲ گرم در کیلوگرم وزن خاک خشک) انجام شد. آزمایش فاکتوریل در قالب طرح کامل تصادفی با چهار تکرار در گیاهان یکساله "مورد" بررسی شد. نتایج نشان داد که تیمار کم آبی (FC٪ ۵۰) به طور معنی داری سبب کاهش شاخص های رشد و همچنین افزایش میزان پرولین، فعالیت آنزیم های آنتی اکسیدانی، و فنول کل در مقایسه با گیاهان خوب آبیاری شده (FC٪ ۱۰۰) شد. در حالی که پروتئین کل محلول، فلاونوئیدها و ظرفیت آنتی اکسیدانی کل (TAC) با افزایش تنش کم آبی نسبت به شاهد تغییر معنی داری نداشتند. SAP افروده شده (۲ گرم در کیلوگرم) باعث افزایش حدود ۴۳/۸۱٪ طول ریشه در مقایسه با تیمار بدون SAP در ۱۰۰٪ FC شد. افزون بر این، استفاده از SAP (۲ گرم در کیلوگرم خاک) به طور قابل توجهی میزان پرولین و آنزیم های آنتی اکسیدانی به ویژه کاتالاز (CAT) را در شرایط کم آبی با فراهم کردن آب بیشتر و کاهش گونه های اکسیژن فعال (ROSS) افزایش داد. نتایج نشان داد که گیاهان جوان "مورد" با افزایش ترکیبات آنتی اکسیدانی و تنظیم کننده های اسمزی مانند پرولین، سازگاری بالایی با محیط های با شرایط نامطلوب دارند. افزون بر این، آن ها قادر به تولید سر آغازه های جدید شاخه با کاربرد SAP در هر دو شرایط آبیاری خوب و تنش های کم آبی می باشند. از این رو، می توان با افزودن SAP به محیط ریشه گیاهان جوان چوبی با روشه کم هزینه و کار، زندگانی و بازده عملکرد آن ها را در مناطق کم آب افزایش داد.

**واژه های کلیدی:** آسکوربیت پراکسیداز، پرولین، ظرفیت کل آنتی اکسیدانی، فنول، کاتالاز