

Full Length Research Paper

The interaction between drought stress and salicylic and ascorbic acids on some biochemical characteristics of *Satureja hortensis*

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Water is one of the most important environmental factors that regulate plant growth and development, and water deficit is considered the most important restricted factor for plant products, in that several chemical materials have been used to reduce the harmful effects of water deficit. Some of these compounds (for instance, ascorbic and salicylic acid) have antioxidant effects in plants. In order to study the interaction between drought stress with salicylic and ascorbic acid on some biochemical criteria of *Satureja hortensis*, a factorial experiment with a completely randomized design in three replications and different combination of three levels of irrigation (1/3, 2/3 and total field capacity), three levels of salicylic acid (0, 1 and 3 mM) and three levels of ascorbic acid (0, 1 and 3 mM) under green house conditions was conducted. Results, revealed under aridity, sugar content and protein content of *S. hortensis*, were decreased; however, proline and lipid per oxidation increased. The results concluded that when drought stress affected the plants, the protective mechanism was activated. The reduction of protein content in this condition showed that drought stress affected protein synthetic and degradation. The reduction of sugar content showed that stress affected the photosynthetic process. On the other hand, in the presence of both salicylic and ascorbic acids, the harsh influences of water deficit reduced and some growth parameters increased. It seemed that two external acids were able to enhance the tolerant ability of the plant to aridity stress.

Key words: *Satureja hortensis*, water deficit, salicylic acid, ascorbic acid.

INTRODUCTION

Water is one of the most abundant compounds on the ground and 2/3 of the ground-level was surrounded by water, but in most part of the world, lack (shortage) of water is a factor which is limit the production of the agricultural products (Redy et al., 2003). Lack (shortage) of water in Iran is accounted as a factor which limit the plantation and growth of the pharmaceutical and agricultural plants. Drought stress in the different stages of growth during flowering and grading stages is a factor for limiting the performance. It seemed that the pharmaceutical plants have various reactions to the

drought stress on the performance and effective productive materials (Hissao, 1973). For understanding these features, doing widespread researches on the pharmaceutical plants and performing the different treatments are also needed. The lack of humidity (shortage of it) forced the plants to the different morphological reactions such as reduction in the leaf-surface, early-fall, reduction of the aerial organ, development of the root-growth, physiologically and metabolically reactions such as closing the pores, reduction in the growth rate, solution substances, the operation of the different genes etc. Drought stress is accounted for as a main factor in the environmental stresses. Cold stresses, freezing, warm, wind and salt are associated with drought stress and they injured the

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plants through this way.

Response to the water stress is related to the other factors such as duration, the intensity of stress, the developmental stage or growth of the plants. The determined metabolically and physiological responses of agricultural plants to the drought stress condition were studied, but for the pharmaceutical and aromatic plants under the lack of humidity are not known. Drought stress affects the different stages of photosynthesis. Drought stress effects on the photo system II and the proteins of chloroplast estromai as Robisco decreases the photosynthesis (Inze and Montago, 1995). Drought stress will decrease and decompose the chlorophyll (Sairam et al., 1998). In this condition (shortage of water), the ability for opening the pores was quietly decreased, and when the intense water stress happened, the pores did not respond to the outer (external) factors and would remain as the close pores (Borsani et al., 2001). Drought stress will affect the lipids' per oxidation, proteins' metabolism and also the plants' growth regulators (phytohormones). Considering these reasons (such as reduction of annual rainfall and commencement to termination of the underground water sources), the pressing needs of plants to the water and the injurious effects which resulted from drought stress and drought, forced the researchers to decrease the injurious effects by adding some compounds. Salicylic and ascorbic acids are two main compounds in the plants which play an important role in decreasing the effects of drought stress. Considering the increasing importance of drought stress in the growth of plants especially pharmaceutical ones, the effect of the drought stress on some of the physiological features of pharmaceutical plants such as *Satureja hortensis* and also the effects of ascorbic and salicylic acids on the reduction of the amount of drought stress effect on the aforementioned features were studied. Ascorbic acid is the best well known compound used for antioxidants and is necessary for collagen synthesis. It is the most effective compound which increases the tolerance of the plants to oxidative stresses.

Ascorbic acid plays an important role in photosynthesis and also has a role in the defense against oxidative stress. The recent results, using the transgenic plants and mutants, confirmed the role of ascorbic acid and the glutathione cycle of ascorbic acid in oxidative stress, but in the plants under stress, the amount of ascorbic acid was also increased. Salicylic acid was the organic compound in the plants and it affects the physiological activities in the plants and was considered as an important one in the drought stress resistances. The most important roles of salicylic acid are included in its effect on seed budding, growth, fruit maturity, flowering and the responses to stresses. Also, salicylic acid considerably affects the membrane structure and photosynthesis system. The greatest amount of salicylic acid was found between the testing plants such as rice, barely and soya

and it was shown that 1 µg/g is their wet weight (Raskin, 1992). *Satureja hortensis* is the plant which belongs to the mentha (*Lamiaceae*) family. It is a grassy and annual plant and it has a branched stem with 10 to 30 cm heights whose color is darker than its leaf. On the leaf surface, we can see the small and abundant spots, termed as a gland, and which contained essence. The *Satureja hortensis's* small white and colorful flowers which are blossoming in the summer are put on the length of the stem branches, collectively. The pharmaceutical benefits of this plant point to the flowered head branch that has a strengthening effect, which facilitate digestion and strengthen stomach and the digestion system, and also it plays an important role in removing the digestion diseases. Dispersion is mostly seen in the north – west of Iran, and also in some parts of Khorasan.

MATERIALS AND METHODS

This study was carried out in order to investigate the effect of salicylic and ascorbic acids on the amount of some compounds of *Satureja hortensis* under drought stress. The experimental design was a factorial experiment, completely randomized in three replications.

Eight seeds were planted in the plastic pots (vases) with 10 cm diameter and 15 cm height which contained soil with the ratios of sand, clay and peat (2:1:1) in greenhouse. In addition, for improving and accelerating the growth of plants, the Hoagland food (nutrient) solution with the dilution of 1 to 3 was added to the pots (vases) weekly. After 7 days from sowing, plants were thinned to 6 plants / pot. It should be noted that light conditions were equal to 14 h light and 10 h darkness, and also the laboratory humidity was equal to 30 to 40% and the temperature was 25°C. The treatments used were as follows:

(A) Irrigation treatments:

1. No stress.
2. 1/3 field capacity.
3. 2/3 field capacity.

(B) Salicylic acid concentrations:

1. Zero.
2. 1 mM.
3. 3 mM.

(C) Ascorbic acid concentrations:

1. Zero.
2. 1 mM.
3. 3 mM.

Before starting the drought stress, the plants were irrigated daily accordingly, till the field capacity was full. Performance of drought stress was done after seed growth and the appearance of the fourth leaf for 18 days at 1/3 and 2/3 field capacities on the plants. At the same time, ascorbic and salicylic acids solutions were applied as foliar spraying. In addition, some of the pots were irrigated in the extent of field capacity as daily evidence.

In this study, after performing the drought stress and using the ascorbic and salicylic acids, the biochemical features, such as: (1)

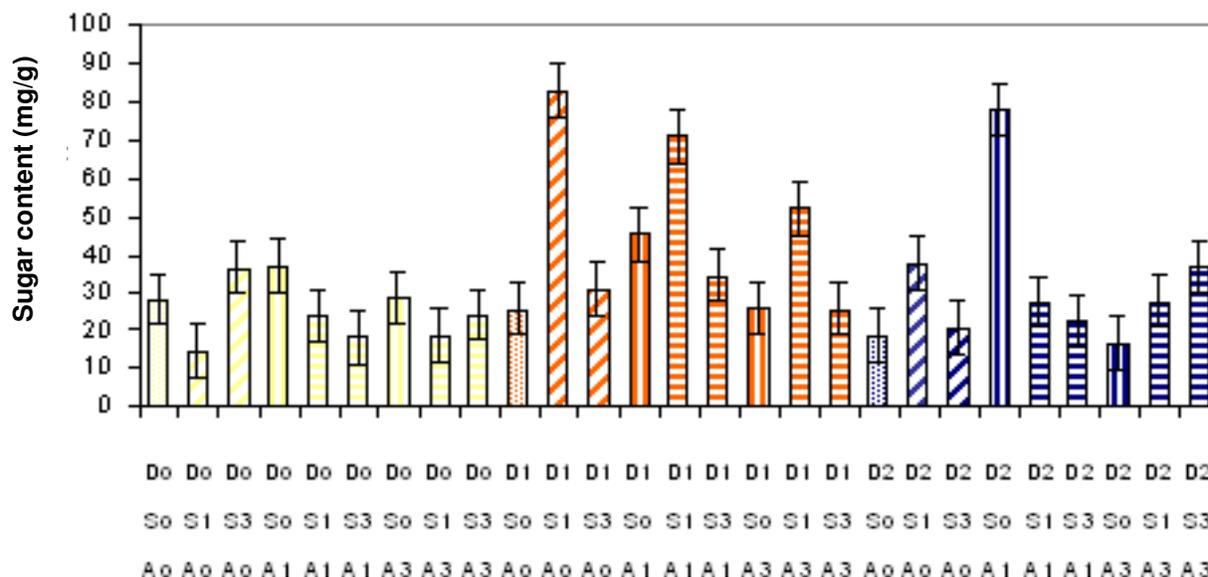


Figure 1. The interaction of drought stress, salicylic and ascorbic acid on the amount of leaf sugar.

proline, (2) sugar, (3) protein and (4) lipids' per oxidation, were measured. Proline content in the leaf tissue was determined according to the method of Bates et al. (1973). About 0.2 g of the leaf fresh tissue was weighted and abraded in china mortar of 10 ml sulfa salicylic acid (3%). The achieved juice was centrifuge in a 10000 G device for 5 min. Then 2 ml of the solution from the centrifuged device with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent (1.25 g ninhydrin acid, 30 ml of glacial acetic acid and 20 ml NH_3PO_4 (6M)) was incubated for 1 h at 100°C. The reaction was stopped by placing the test tubes in cold water. The samples were rigorously mixed with 4 ml toluene. The light absorption of toluene phase was estimated at 520 nm using spectrophotometer. The proline concentration was determined using a standard curve, while the results of measuring the proline content was calculated and presented with $\mu\text{g/g}$. The measurement of revival sugars was done by Somogy in 1952 and the concentration of sugar was obtained on the basis of mg/g. The measurement of all proteins and the amount of proteins in the leaves was measured by Lowry method in 1951 and the concentration of protein was reported on the basis of mg/kg (Lowry, 1951). Then for calculating the content of per oxidation in membrane fats, the concentration of malon- dialdehyde (MDA) from this reaction was measured. The measurement of MDA concentration was done in heat and packer method (1969). According to this method, 0.2 grams of leaf fresh tissue was weighted, and was abraded in a china mortar of 5 ml trichloroacetic acid (TCA) of 0.1%. The achieved juice was centrifuged by a centrifuge device in 10000 $\times\text{g}$ for 5 min. About 5.4 milliliter TCA solution which is 20% and which contains 0.5% thiobarbituric acid (TBA) was added to 1 ml of the solution from the centrifuge. The achieved compound was heated in hot bath at 95°C for 30 min. Then, it became cool in ice immediately and again the compound was centrifuged in 10000 $\times\text{g}$ for 10 min. The intensity absorption of this solution was read by spectrophotometer in a wavelength of 532 nm. The specified compound for absorption of this wavelength is red complex (MDA-TBA). The absorption of other nonspecific pigments was measured in 600 nm and it reduced in this content. For calculating MDA concentration, the silence coefficient was used, and the results from the measurement was calculated and presented according to mgr on fresh weight gram. For measuring the other aldehydes (Propanal, Botanal, Hegzanal,

Heptanal and Propanal Dimethylestal), the Meirs method in 1992 was used. The obtained results from the measurement were presented on the basis of mg/g.

Subsequently, the results were analyzed by the SPSS 14 software and the resulted averages were compared by Duncan test. Therefore, the diagram's (graph) design was done in the SPSS 14 software.

RESULTS

In this part, the effect of the performed treatments on the biochemical parameters such as the amount of sugar, proline, protein and lipids' per oxidation was studied.

For presenting the explanation of the results and the discussion, the abbreviations, such as D for Drought stress (D_0 = natural irrigation, D_1 = 1st level of dry stress and irrigation equal to 2/3 field capacity, D_2 = 2nd level of dry stress and irrigation equal to 1/3 field capacity), A for acid ascorbic treatment (A_0 , A_1 , A_3 = acid ascorbic in the concentration of 0, 1 and 3 mM) and S for acid salicylic treatment (S_0 , S_1 , S_3 = acid salicylic in the concentration of 0, 1 and 3 mM), were used.

Figure 1 is related to the amount of leaf sugar and it shows that the least amount is related to the plants which are placed under the 2nd level of drought stress (irrigation equal to 2/3 field capacity), with 3 mM ascorbic acid ($D_2S_0A_3$) and was performed using the 2nd level of drought stress ($D_2S_0A_0$) without treatment of the given organic acids.

The greatest amount of sugar is related to the plants which are placed under severe drought stress (irrigation equal to 1/3 field capacity), with 1 mM salicylic acid ($D_2S_0A_1$) and was also performed using the 1st level of drought stress (1/3 field capacity) with 1 mM salicylic and ascorbic acids ($D_1S_1A_1$).

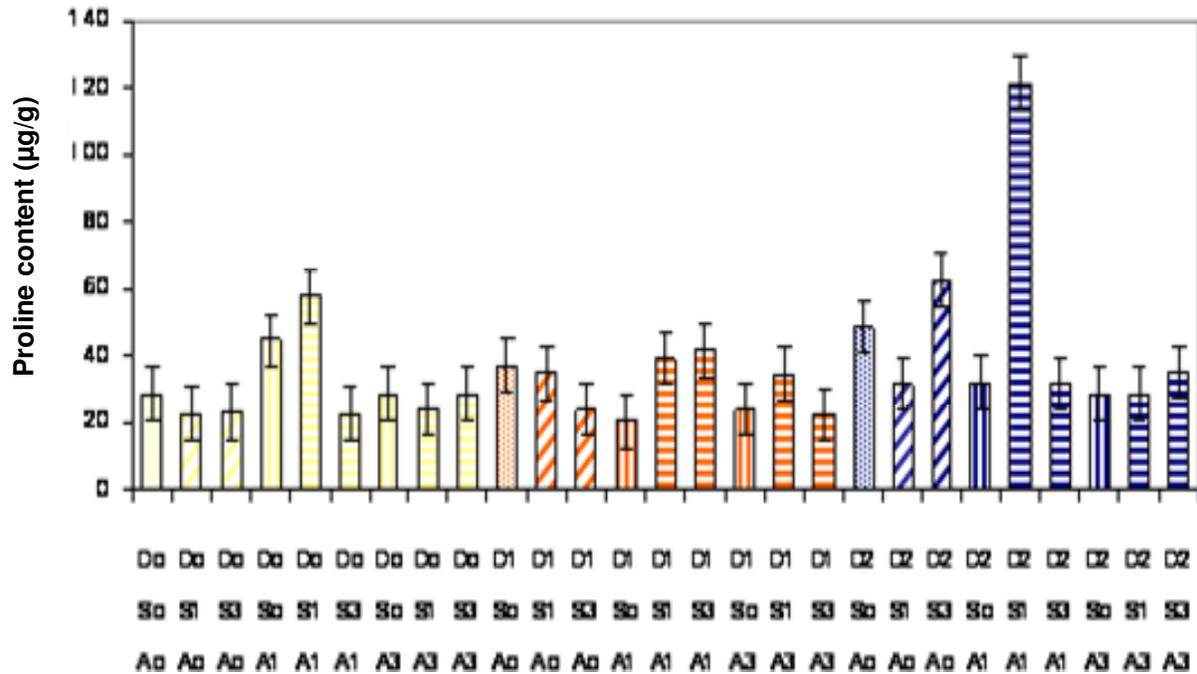


Figure 2. The interaction of drought stress, salicylic and ascorbic acid on the amount of proline.

Figure 1 indicated that drought stress was associated with the sugar amount, and if the level of drought went up, the amount of sugar decreased. This reduction was mainly due to the effect of drought stress on the amount of chlorophyll and photosynthesis. In some conditions where the plants are placed under the 1st level of drought stress (irrigation equal to 2/3 field capacity), in combination with ascorbic and salicylic acids, the concentrations could be associated with the increasing amount of sugar and this effect could lead to the improvement of plant resistance. In general, application of different concentrations of ascorbic and salicylic acids is associated with the increasing amount of sugar, but this increase was only with 1 mM salicylic acid (D₁S₁A₀) and was so great with 1 mM salicylic and ascorbic acids (D₁S₁A₁). Also, this will increase the height of the figure to the evidence group. In the other conditions where the plants were exposed to severe drought stress (irrigation 1/3 field capacity), in combination with ascorbic and salicylic acids, in most cases it was associated with increasing the amount of sugar, while in other cases, it was associated with the decreasing sugar content which was only seen with 3 mM ascorbic and salicylic acids.

In this study, it was observed that if the *Satureja hortensis* plant was involved in drought stress, adding the salicylic and ascorbic acid s(with 1 mM concentration) would be effective for decreasing the resulted damage from the drought stress on sugar and also would increase its amount.

Figure 2 illustrated that the least amount of proline is related to the plants exposed to the mid drought stress

(irrigation equal to 2/3 field capacity) in combination with 1 mM ascorbic and salicylic acids (D₁S₀A₁) and the 1st level of drought stress (irrigation equal to 2/3 field capacity) in combination with 1 mM ascorbic and salicylic acids (D₁S₃A₃).

The greatest amount of proline is related to the plants exposed to severe drought stress (irrigation equal to 2/3 field capacity) in combination with 1 mM ascorbic and salicylic acids (D₂S₁A₁) and 3 mM salicylic acid (D₂S₃A₀).

Figure 2 also illustrated that when the plants were kept under drought stress, the amount of proline would increase. This increase will become so great with an increase in the period of drought stress. On the other hand, when plants were kept under mid drought stress, performing (using) different concentrations of ascorbic and salicylic acids, in some cases, was associated with increasing the amount of proline; then, it improved the plant's resistance toward drought stress. This increase (resistance increase) was seen mostly with 1 mM of ascorbic and salicylic acids, but in the other cases, its performance (using ascorbic and salicylic acids) was associated with the reduction of proline using 1 mM ascorbic acid (D₁S₀A₁), 3 mM of salicylic acid and ascorbic acid (D₁S₃A₃). Severe drought stress (irrigation equal to 1/3 field capacity), in combination with ascorbic and salicylic acids, was mostly associated with the reduction of the amount of proline to the evidence group by using 1 mM of salicylic acid (D₂S₁A₀) or 1 and 3 mM of ascorbic acid (D₂S₀A₃, D₂S₀A₁). On the other hand, using 1 mM ascorbic acid (D₂S₁A₁) and 3 mM of salicylic acid only (D₂S₃A₀) was faced with the augmentation (increasing)

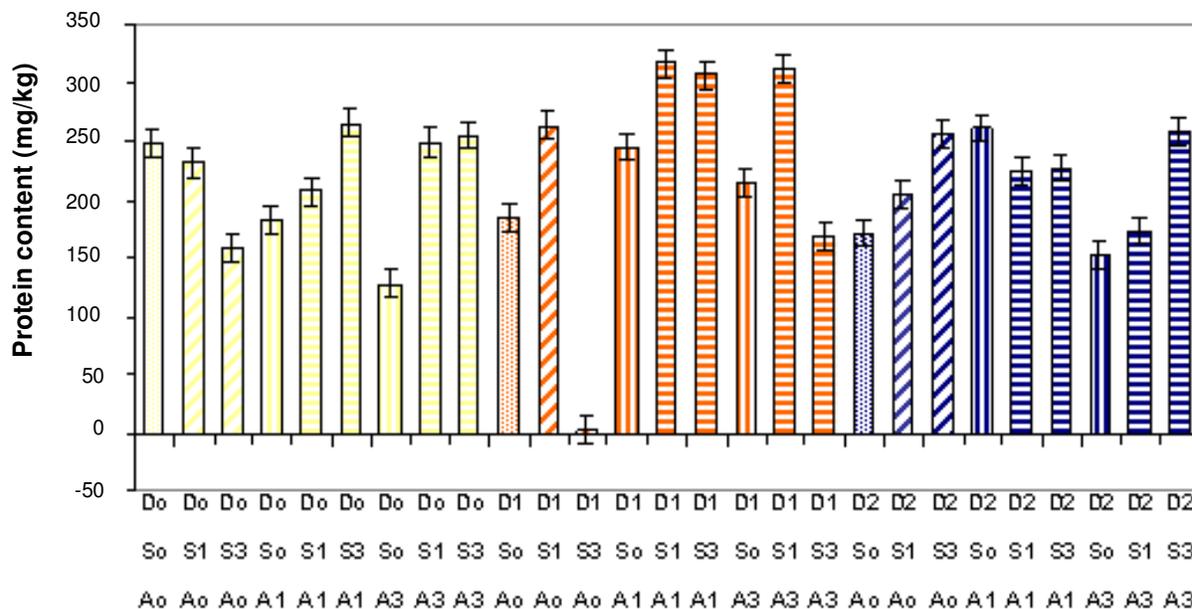


Figure 3. The interaction of drought stress, salicylic and ascorbic acid on the amount of protein.

of this parameter, and also it increases the resistance of the plant.

Figure 3 illustrated that the least amount of protein is related to the plants which were kept under the mid drought stress (irrigation equal to 2/3 field capacity) in combination with 3 mM salicylic acid ($D_1S_3A_0$), and also under the severe drought stress (irrigation equal to 1/3 field capacity) in combination with 3 mM ascorbic acid ($D_2S_0A_3$).

Nevertheless, the greatest amount of protein is related to the plants which were kept under the mid drought stress (irrigation equal to 1/3 field capacity) in combination with 1 mM ascorbic and salicylic acids ($D_1S_1A_1$) and also the 1st level of drought stress in combination with 1 mM salicylic acid or 3 mM ascorbic acid ($D_1S_1A_3$).

Figure 3 illustrated that the drought stress was associated with the reduction of the amount of protein and this reduction was so great in the 2nd level of drought stress than the 1st level of drought stress and the evidence plants. In some plants which were kept under the mid drought stress, performing (using) the second compounds (acid ascorbic and acid salicylic) in different concentrations was associated with increasing the amount of protein, and this increase was so great by using 1 mM of ascorbic and salicylic acids ($D_1S_1A_1$), 3 mM of salicylic acid, 1 mM of ascorbic acids ($D_1S_3A_1$) and 1 mM of salicylic acid or 3 mM of ascorbic acid, but in the other cases, performing (using) 3 mM of salicylic acid only ($D_2S_3A_0$), has the greatest reduction effect on the amount of protein. About the plants which were kept under the severe drought stress (irrigation equal to 1/3 field capacity), in combination with 1 mM ascorbic and salicylic acids (especially with 1 mM of ascorbic acid, 3 mM of salicylic acid or in combination with 3 mM ascorbic

and salicylic acids), performance was associated with increasing the amount of protein, and consequently, it removed the effect of drought stress which was the reduction of the amount of protein.

Figure 4 illustrated that the least amount of lipids' per oxidation (MDA) is related to the plants which were kept under the suitable irrigation treatment ($D=0$) in combination with 1 mM ascorbic and salicylic acids ($D_0S_1A_1$), and also, it is related to the suitable irrigation treatment under 3 mM salicylic acid or 1 mM ascorbic acid ($D_0S_3A_1$).

The latest order is about the treatment group of the 1st level of drought stress and 3 mM salicylic acid or 1 mM ascorbic acid ($D_1S_3A_1$). The greatest amount of lipids' per oxidation were related to the severe drought stress in combination with 1 mM of ascorbic acid ($D_2S_0A_1$) and also at the mid drought stress with 1 mM of ascorbic acid ($D_1S_0A_1$).

Figure 4 also indicated that drought stress increased the lipids' per oxidation in the *Satureja hortensis*. In the plants which were kept under the mid drought stress (irrigation equal to 1/3 field capacity), in combination with ascorbic and salicylic acids, in some cases, it was associated with increasing the amount of this parameter. This increase, mostly, will appear with 1 mM ascorbic acid ($D_1S_0A_1$) or 1 mM salicylic acid and 3 mM ascorbic acid ($D_1S_1A_3$), but the greatest reduction effect on the lipids' per oxidation was noticed at mid drought stress with 3 mM of salicylic acid or 1 mM ascorbic acid, in which the height of the table considerably decreased.

The plants which were kept under drought stress equal to 1/3 field capacity was like this. In most cases, adding ascorbic and salicylic acids was associated with decreasing the amount of MDA and only the increasing or

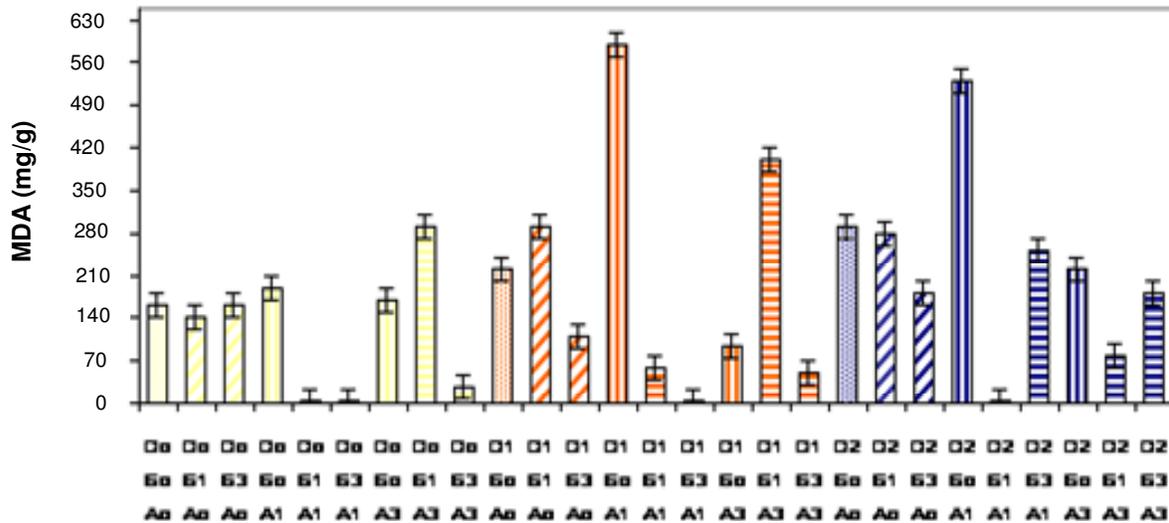


Figure 4. The interaction of drought stress, salicylic and ascorbic acid on the amount of MDA.

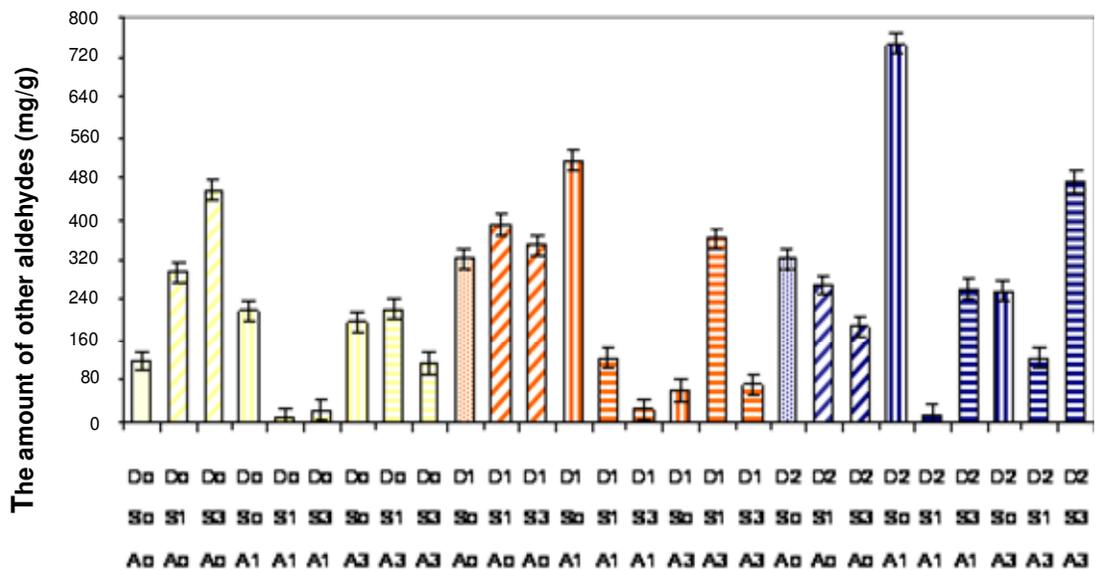


Figure 5. The interaction of drought stress, salicylic and ascorbic acid on the amount of the other aldehydes.

augmentation effect was recorded by using 1 mM ascorbic acid. Also, 1 mM ascorbic and salicylic acids became less and was nearly set to the x - axis. In this study, it was observed that drought stress can be damaged by adding ascorbic and salicylic acids.

Figure 5 illustrated that the amount of the other aldehydes was considerably increased as the result of drought stress and this was shown that the production of free radicals due to drought stress was so great than the amount of membrane resistance (tolerance). Finally, it would damage the membrane, but the drought stress in combination with ascorbic and salicylic acids decreased the amount of aldehydes and it is indicative of the plant resistance to drought stress. However, the greatest effect

of most of the performed treatments is so similar to the effect of MDAs.

DISCUSSION

Based on the experiments in this study, it was observed that the drought stress was associated with the reduction of the amount of sugar and if the level of dryness increases, the amount of sugar will decrease (Figure 1). The reason for this reduction is the effect of drought stress on the amount of chlorophyll and photosynthesis. In this study, one of the reasons for decreasing the amount of carbohydrates in the leaves of the plants under

the drought stress condition resulted from the effects of these stresses on the membrane of Thylacoides, the amount of photosynthesis pigments and also the amount of photosynthesis. Thus, the concentration of the solution sugars was observed in the plants species.

In the leaves of rapeseed, onion and peanut (Redy et al., 2003), beet, potato (El-Tayeb, 2005) and its cells, drought stress leads to the augmentation of (increasing) the starch resolution and the concentration of solution sugars (sucrose and fructose) which help to maintain the cellular inflammation. It was reported that the drought stress caused many changes in the amount of plant's carbohydrate and it was determined that by increasing the drought stress in the leaves, the amount of starch in them will decrease. The reduction of the sugar content could be as a result of photosynthesis reduction, because the reduction of water leads to the reduction of turgor and also reducing the turgor pressure would lead to the closeness of the pores and finally would decrease the photosynthesis.

In this study, when the plants were kept under drought stress, the amount of proline increased and this increase became so great by exceeding the drought stress. When the plant was exposed to the mid drought stress, performing (using) the ascorbic and salicylic acids at different concentration in some cases was associated with increasing the amount of proline (Figure 2). As such, the plant's resistance would be improved against the drought stress.

Redy et al. (2003) have reported that the amount of proline in the drought stress time would increase, in that amino acid proline is a key in osmosis regulation. In the investigations by Sairam et al. (1998), they reported that increasing the proline would lead to increase in the resistance of salt stress. In different varieties, the amount of this augmentation or increase is variable and the source of proline is the entire protein or amino acid. Studies showed that in the callus plant, *Pinus taeda*, under the severe drought stress, the concentration of proline will increase 40 times. It means that when this plant (*Pinus taeda*) was placed under severe drought stress, the concentration of proline increased 40 times.

Ascorbic and salicylic acids would affect the metabolism of plant reactions and would lead to many changes in them. These changes are sometimes accounted for as adaptabilities which increase the tolerance or resistance of plants against the environmental factors (Metwally et al., 2003). On the other hand, it was reported that increasing (development of) proline will cause the preservation of (keeping) the cellular inflammation and reduction of the membrane damage in plants, so the osmosis regulation is as an adaptability which increases the plant tolerance or resistance to drought stress (Inze and Montago, 1995).

In plants such as barely, wheat, bean and tomato, during the oxidative stress, the amount of proline and sugars concentration were increased by the treatments of

salicylic acid hormone. Increasing the amount of proline, sugars and also the osmosis gradient in the plants would lead to the resistance against losing water, the contents of leaves and also accelerate the growth of plants in stress conditions (Tasgin et al., 2006).

It was noted that the concentration of proline has a direct and positive relationship with increasing the created resistance in the plants exposed to non-biological stress (Ramanjulum et al., 1998).

In this study, drought stress was associated with decreasing the amount of protein, and this reduction (decrease) was so great in the 2nd level than the 1st level and the evidence plants. In the plants which were kept under the mid drought stress, performing (using) the second compounds (ascorbic and salicylic acids) in different concentrations, in most cases, was associated with increasing the amount of protein (Figure 3). For explaining this issue, we could say that under the unsuitable environmental conditions such as drought stress, the active species (types) of oxygen would produce and concentrate, and consequently, the increasing H₂O₂ would lead to increase in the oxidation of proteins in several types of plant species. Drought stress is a factor used to decrease the Robisco activity and also its amount in the plant. Drought stress lead to the reduction (decrease) of protein formation (composition) in several types of plant species through decreasing the cellular polysoms, and it was determined that the drought stress caused the activation of the protective genes in the plants, and consequently, it increased the amount of HSP protein. On the other hand, it caused the formation of the important enzymes such as Robisco, phospho phroktokinas, invertas and sacaros synthetas in the aerial organs in drought stress conditions (Inze and Montago, 1995).

The radicals of active oxygen facilitated the effects of decomposition enzymes of proteins by changing the situation of amino acids in the protein branches (strings), and therefore one of the most important reasons for decreasing the content of protein in the plants exposed to drought stress is the production of oxygen free radical (Somogy, 1952).

It was reported that salicylic and ascorbic acids would affect the defensive proteins, types of kinase protein and Robisco, and also it was proven that salicylic acid infused the synthesis of the controlled proteins of plant or herbal proteases (Popova et al., 1997; Raskin, 1992).

Finally, El-Tybe (2005) reported that the content of solution protein and free amino acids in the aerial organs and the root of seedlings under the stress conditions were reduced.

Salicylic acid caused the augmentation of antioxidant enzymes in the plants such as bean and tomato (Vera and Conejero, 1990). Therefore, the reduction of the protein content or increase in its resolution (decomposition), or both of them were attributed to the increase in the activity and the anti oxidant enzymes.

Salicylic and ascorbic acids protect the protein oxidation by increasing the potential of antioxidants (Sairam et al., 1998).

In measuring the lipids' per oxidation, it was observed that drought stress caused the augmentation (increasing) of lipids' per oxidation in *Satureja hortensis* (Figures 4 and 5). The cellular membrane and the other internal membranes (chloroplast membrane and mitochondrial) were formed from two layers of phospholipids. The produced super oxide radicals from the drought stress caused the lipids' per oxidation (Sarema et al., 1998; Sarkar and Tophan, 1974; Dewlin and Withman, 2002).

The production of membrane lipids' per oxidation would be some compounds such as MDA, propanol, botanal, hexanal, heptanal and propanal de methylastal and these compounds were used as an index for measuring the amount of lipids' per oxidation. Increasing the lipids' per oxidation was considered as an index for increasing the oxidative stress (Heat and Pacher, 1969; Meirs et al., 1992).

In this study, the drought stress increased the amount of MDA and the other aldehydes. There were many reports based on drought stress on the amount of MDA and the other aldehydes.

Sairam et al. (1998) reported that the amount of MDA increased in three wheat genotypes under drought stress.

It was proved that the amount of MDA - productive during the drought stress between the different cultivars of maize, banana and rice seedlings is so different. The resistant species are capable to sweep H_2O_2 by increasing the anti oxidant ability but by decreasing H_2O_2 , the less amount of MDA would be created. Thus, the amount of the sensitive MDA - productive is so great (Sharma and Shanker, 2005; Inze and Montago, 1995; Li et al., 1998).

Loggini et al. (1998) declared that drought stress did not affect lipids' per oxidation in wheat cultivars.

However, Fu and Haung in 2001 observed that in the mid drought stress, the amount of MDA increased and the other aldehydes increased in two plants of the cold region, *Poa pratensis* and *Festuca* (Fu and Haung, 2001).

In this study, the observed increase of MDA and the other aldehydes in the dry conditions resulted from producing the active oxygen species such as super oxide radical, peroxide, hydrogen and radical hydroxide, which is in the oxidative stress condition. The species of active oxygen leads to lipids' per oxidation as a result of injury or damage to the cellular membrane, especially chloroplast membrane. There were many reports about role salicylic acid in removing the stress of per oxidative, but in recent years, the roles of salicylic acid on water and non-water stresses were completely studied. However, salicylic and ascorbic acids, affecting the anti oxidant enzymes and lipids' per oxidation, would protect the Arabidopsis plant against warm and drought stress (Larkindale and Knight, 2002).

Studies showed that salicylic acid prevented the damages to the unsaturated fatty acids, while the penetration of the membrane reduced the protection of the thilacoid membrane in salt stress duration in Arabidopsis seedling (Borsani et al., 2001).

In addition, the production of MDA salicylic acid during stress decreased the salt in the leaves and roots of barely plants (Elstner et al., 1976).

In contrast, there were so many reports based on the reduction of the anti oxidant ability and consequently the development of (increase) the free radicals in the salicylic acid treatments (Liusia et al., 2005).

Metwally et al. (2003) proved that treatment of barely seedlings with cadmium through the activation of anti oxidant system would decrease the amount of MDA and the other aldehydes which were obtained from the lipids' per oxidation of the membrane fats to the plants which were kept under salt stress (Metwally et al., 2003).

In this study, it was determined that salicylic and ascorbic acids or augmentation of lipids' per oxidation which arose from drought stress were reduced. Therefore, acid salicylic and acid ascorbic protected the *Satureja hortensis* plant against the oxidative stress by decreasing the lipids' per oxidation through its affect on the defensive mechanism of enzymes. Meanwhile, there were many evidences based on this issue that acid salicylic and acid ascorbic increased the anti oxidant potential by affecting H_2O_2 . Also, it protected the plants against oxidative stresses (Ganesan and Thomson, 2001; Davis, 2005).

In contrast, there were many reports based on the salicylic acid effect on the ACC synthetase and ACC oxidase (Zhu, 2001).

Therefore, salicylic and ascorbic acids decreased the lipids' per oxidation by affecting the amount of productive H_2O_2 (Ganesan and Thomson, 2001).

Based on the obtained results in this study, there is a close relationship between drought stress, salicylic and ascorbic acids in activating the lipids' per oxidation in a way that it will have double effects on salicylic acid. Also, the different receptors of this compound were added to the complexity of this way. For confronting the infused oxidative stress by the plants drought stress, the different defensive mechanisms such as enzymatic or non-enzymatic mechanisms were used. One of the non-enzymatic mechanisms is acid ascorbic. Increasing the amount of ascorbate in drought stress is indicative of this issue that the plants would lead to a removal of the poison from the species of the activated oxygen by activating the ascorbic cycle (Dewlin and Withman, 2002).

The observed increase in the linked treatment with acid salicylic caused the plants' adaptability changes toward stress.

The obtained results are similar with the other results by the researchers about the effects of drought stress on the growth of the plants:

1. In this study, the amount of *Satureja hortensis* sugar decreased due to the drought stress which has the greatest downward movement in the 2nd level of the dry region. One of the reasons for decreasing the amount of carbohydrates in the plants' leaves under drought stress conditions is as a result of the stress effect on the membrane of thylacoids, the amount of photosynthesis pigments and also the amount of photosynthesis. The reduction in the amount of sugar could be the result of the amount of photosynthesis, because the reduction in water could lead to the reduction of the cellular inflammation, losing the turgor pressure, closing the pores and finally reducing the photosynthesis. In the linked treatment with salicylic and ascorbic acids, especially in 1 mM concentration of these compounds, the amount of sugar increased.

2. The data obtained indicated that there was an increase in the amount of proline. The increase of proline could be the reason for keeping the cellular inflammation and reduction of the membrane's damage in the *Satureja hortensis*. Osmosis regulation is accounted for as an adaptability which increased the plant tolerance or resistance to the drought stress. Salicylic and ascorbic acids would affect the plants' metabolism on reactions and would cause so many changes in them. These changes are accounted for as adaptabilities which increase the amount of plants' tolerance or resistance and also their adaptability toward the environmental factors. Among the performed (used) concentrations from the organic acids, in the mid drought stress, 3 mM of salicylic acid and 1 mM of ascorbic acid increased the amount of proline and in the severe drought stress, 1 mM of these compounds increased the amount of prolix and caused the great tolerance or resistance of the plant against the drought stress.

3. In this study, under the drought stress condition, the content of the leaves' plant protein also decreased in the evidence plants. In explaining this issue, it should be mentioned that the radicals of active oxygen facilitated the effect of decomposition enzymes of proteins by changing the amino acids situations in the proteins' strings. Therefore, one of the reasons for decreasing the amount of proteins in the plants exposed to drought stress is producing the oxygen free radicals. In this study, it could increase the amount of protein by the linked treatment of salicylic and ascorbic acids, especially in 1 and 3 mM from these two compounds.

4. In this study, the amount of lipids' per oxidation (the index of oxidative stress) due to drought stress increased and the production of the free radicals was shown under drought stress. Since these radicals were so great than the defensive capacity, they damaged the biological membrane. While using the salicylic and ascorbic acids treatments, the augmentation (increase) of drought stress decreased as well. Therefore, salicylic and ascorbic acids protected the *Satureja hortensis* against (toward) oxidative stress by decreasing the lipids' per oxidation

through its effect on the defensive mechanisms of enzymes.

The obtained results indicated that *Satureja hortensis* could be the resistant plant toward drought stress by using the effective solutions for responding to drought.

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